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XXII. STUDIES ON CARBONIC ACID COMPOUNDS AND HYDROGEN ION ACTIVITIES IN BLOOD AND SALT SOLUTIONS.

A CONTRIBUTION TO THE THEORY OF THE EQUATION OF LAWRENCE J. HENDERSON AND K. A. HASSELBALCH.

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From the Laboratory of the Finsen Institute (Copenhagen).

(*Received March 3rd, 1922.*)

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INTRODUCTION

K. A. Hasselbalch in 1916 introduced a formula with the help of which it is possible to determine the hydrogen ion concentration of the blood from the amount of its combined and dissolved carbonic acid, basing his equation upon the work of L. J. Henderson carried out from 1908–1910. Henderson employed the then generally known equation for the first dissociation of carbonic acid, namely,

$$\gamma \frac{C_H \cdot C_{HCO'_3}}{C_{H_2CO_3}} = K_1.$$

Applied to the determination of the $C_{H^{\cdot}}$ of the blood he got

$$C_{H^{\cdot}} = \frac{C_{H_2CO_3}}{C_{HCO'_3}} \times \frac{K_1}{\gamma}.$$

By substituting the best known values at the time for combined and dissolved CO_2, for the first dissociation constant of carbonic acid and for the dissociation of bicarbonate, he found a value for $C_{H^{\cdot}}$ which corresponded fairly well to the reaction of the blood as it was thought to be at that time. Henderson [1909–1910] concluded from his calculations that the major portion of combined carbonic acid in the blood was in the form of bicarbonate.

Hasselbalch [1916] determined in the same sample of blood (and serum) $C_{H^{\cdot}}$, $C_{HCO'_3}$ and $C_{H_2CO_3}$, and found that $C_{H^{\cdot}}$ was the same as calculated by substituting in the equation the value of $\frac{K_1}{\gamma}$ for a solution of pure sodium bicarbonate. He thought he could infer from this that all the combined carbonic acid of the blood was in the form of bicarbonate and that the $C_{H^{\cdot}}$ of the blood could be estimated by means of the equation when $C_{H_2CO_3}$ and $C_{HCO'_3}$ were known.

As investigations on kindred subjects progressed it became clear the theory could only be recognised as a first approximation—a view which a knowledge of S. P. L. Sörensen's *Studies on Proteins* [1915–1917] strongly supported—and the question immediately arose, how is it possible the calculated and experimental values of $C_{H^{\cdot}}$ can coincide when the suppositions of the calculation are inadequate?

It further became evident from experience gradually accumulated regarding the $C_{H^{\cdot}}$ measurements by the Hasselbalch-Michaelis principle (shaking method combined with minimal immersion), that experimental difficulties were present which had not been entirely overcome, since measurements with various electrodes gave constant differences. And as the Henderson-Hasselbalch equation seemed to be destined to play an important rôle in physiology and pathology it was necessary to subject both the measurements and the theories which formed the basis of the equation to a further test.

The present work therefore has a double object, viz.:

(1) to investigate whether the Henderson-Hasselbalch equation gives correct results when the measurements are carried out by another method than that of Hasselbalch;

(2) to develop a theory connecting the amount of carbonic acid in the blood with its reaction.

In connection with this a number of points have naturally arisen concerning the distribution of ions between blood cells and serum, and a series of experiments on the reactions in simple watery bicarbonate solutions in equilibrium with different tensions of CO_2 have been made.

CHAPTER I

SOME GENERAL PHYSICO-CHEMICAL CONCEPTIONS RELATING TO THE NEW ELECTROLYTIC DISSOCIATION THEORY OF BJERRUM.

Osmolar Concentration.

By the molecular or molar concentration of a solute in a solution we understand the number of gram-molecules of solute in a litre of the solution. We also speak of the molar concentration of the ions and molecular aggregates, looking upon the particles in question as if they were molecules when determining the gram-molar weight. In physiology a distinction has often been made between molecular and molar concentrations (cf. Hamburger [1902], Hedin [1915], Ege [1920]), by imagining all solutes to exist in molecular form (thus Na'Cl' as NaCl) when reckoning the total molecular concentration—a conception which is also made use of in this work in stating the molecular concentration—while in estimating the molar concentration the solute is considered to be in its actual state of aggregation. This distinction has been of especial significance in physiology for questions of osmotic pressure as it is the total number of dissolved particles that is the determining factor (corrected for secondary effects), but as this terminology is not recognised in physical chemistry it must be abandoned. In place of the molar concentration of some physiologists I have therefore employed the expression *osmolar concentration.*

Activity of a Solute.

In 1908 G. N. Lewis formed the conception activity. As regards the thermodynamical definition of this conception I shall confine myself to referring to the article of Lewis [1908], however I shall here quote slightly modified the very perspicuous explanation which Lewis gives in the paper mentioned.

The activity of an ideal gas is equal to the concentration of the gas.

The activity of a solute which forms an ideal solution is equal to its concentration[1].

If a substance has the same activity in two phases, the substance will not by itself go from the one phase to the other.

If the activity of a substance is greater in one phase than in the other, the substance will go from the first phase to the second as soon as it is possible.

By the *activity coefficient* of a solute we mean the factor the concentration of the solute must be multiplied by to give its activity. For uncharged mols in aqueous solution the coefficient will nearly always be greater than unity as it is then merely an expression for what we call depression of solubility, salting out effects, action of salt, etc. For charged mols, "ions," in weak aqueous solutions the coefficient will be less than unity, in strong solutions often greater than unity, as will shortly be shown in discussing electrolytic dissociation. Following Bjerrum [1916, 1918, 1919], we distinguish

[1] Lewis writes ...is by constant temperature and pressure proportional to the concentration.

between the apparent and true activity coefficients, the apparent being the relation between the activity of the desolvated solute and the concentration of the solvated solute, while the true coefficient eliminates solvatation effects. In so far as we do not know whether a solute is solvated we will use the expression *apparent activity coefficient.*

Solubility and Activity of Gases.

In the case of a solution of *the ordinary gases* at room temperature (and low pressure), the activity of the gas can be estimated by determining its tension in a gaseous phase in equilibrium with the solution. This will be understood from what is said later. The gases mentioned in this work, oxygen, hydrogen, carbon dioxide and nitrogen, for the ranges of pressure and temperature used, can in practically every respect be regarded as ideal[1]. The tension of such a gas will thus be a direct measure of its activity. For instance if Henry's law applies, the amount of gas which is *dissolved* in a solution in a state of equilibrium will be proportional to its pressure. The factor which expresses the amount of gas dissolved at a given pressure is called the *absorption coefficient.* Bunsen's absorption coefficient is used here which is called α, and which gives the number of cc. of gas reduced to *0° C. and 760 mm. Hg,* that are dissolved in 1 cc. of solution at a pressure of 760 mm. of the gas itself. At a pressure of P mm. Hg, a cc. of gas will then be dissolved in 1 cc. of the solution

$$\frac{Pa}{760} = a \quad \text{.....(1)}$$

That we use Bunsen's absorption coefficient and not Ostwald's, is because we want to estimate the concentration of the gas in the solution and the former way of expressing it is most suitable for this purpose. A gram-molecule of an ideal gas at 0° C. and 760 mm. Hg occupies a volume of *22393 cc.* A solution containing a cc. of gas (0° C. and 760 mm. Hg) in 1 cc. will contain 1000 a cc. per litre and the gas will therefore have a concentration of

$$\frac{1000a}{22393} = 0{\cdot}044656 \times a = C_{\text{ideal gas}}. \quad \text{.....(2)}$$

Expressing the dissolved gas in volumes per cent. (Vols. %) this will be equal to 100 a and the concentration will be

$$0{\cdot}00044656 \times (100\,a) = C_{\text{ideal gas}}. \quad \text{.....(3)}$$

The constant 22393 is valid for an ideal gas; for CO_2 the corresponding constant is *22263*[2]. The difference between these two values is a little over $\frac{1}{2}$ % (the deviation of CO_2 from Avogadro's law at 0° C. and 760 mm. Hg). The last constant is of course used for the calculation of the concentration of CO_2.

For *carbon dioxide* therefore we have the corresponding equations

$$0{\cdot}044917 \times a = C_{CO_2} \quad \text{.....(4)}$$

and

$$0{\cdot}00044917 \times (100\,a) = C_{CO_2}. \quad \text{.....(5)}$$

[1] An ideal gas obeys the "gas laws."

[2] These constants are calculated according to F. W. Küster, *Logarithmische Rechentafeln für Chemiker u.s.w.* 19th edition, Leipzig, p. 40, 1918.

If we desire to express the concentration in terms of the absorption coefficient and the tension we have, for an ideal gas,

$$P\alpha \times 0{\cdot}000058756 = C_{\text{ideal gas}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

and for CO_2

$$P\alpha \times 0{\cdot}000059101 = C_{CO_2}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

Activity Coefficients and Depression of Solubility of Gases.

We will now consider the value α a little more closely. Equation (1) $\frac{P\alpha}{760} = a$ is valid without exception for pure solvents. In this work we shall assume the same is the case for all solutions if the correct absorption coefficient is used but it must be mentioned there may be small deviations in heterogeneous solutions. If a gas dissolves in a pure solvent,

$$C_{\text{gas}} = A_{\text{gas}} \text{ (Activity of the gas).} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(8)$$

If other solutes are already present in the solvent a correction must be introduced. In such solutions the absorption coefficient is

$$\alpha_{\text{corrected}} = \frac{100 - Y}{100} \times \alpha \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(9)$$

and the concentration of the gas will then be

$$P\frac{100 - Y}{100}\alpha \times k = C_{\text{gas}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(10)$$

while the activity of the gas is

$$P \times \alpha \times k = A_{\text{gas}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(11)$$

and

$$C_{\text{gas}} \times \frac{100}{100 - Y} = A_{\text{gas}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(12)$$

Y is called the *relative depression of solubility*[1],

$\frac{100 - Y}{100}$ is called the *relative absorption coefficient*, and

$\frac{100}{100 - Y}$ is the *apparent activity coefficient* of the gas.

It will be noticed that the apparent activity coefficient is the reciprocal of the relative absorption coefficient. The apparent activity coefficient for a gas is called F_a (gas), the value α is reserved for the absorption coefficient in the pure solvent.

For watery solutions the relative absorption coefficient has been determined in a large number of experiments, in different ways, for numerous gases, and it has been shown that its value in a given solution is about the same for all gases.

The relative absorption coefficient has also been determined for various solid substances which we have reason to think do not unite with other solutes, by estimating the partition of the substance between the solution and a liquid which does not mix with the latter and in which the solute under investigation may be assumed to form a "true" solution. It has been found the same laws apply as for gases.

[1] This applies to homogeneous solutions, whilst the symbol Ψ is used for this quantity in a heterogeneous solution.

Lastly it has been discovered that the relative absorption coefficient, within the experimental error, is independent of temperature. The solutes that most markedly depress solubility in water are the electrolytes, but non-electrolytes can also have a large effect, *e.g.* cane sugar. There is an extensive literature on the solubility of gases in water which is exhaustively dealt with in Rothmund's monograph [1907] and cited in Landolt and Börnstein's tables, to which the reader is referred.

Bohr and Bock's [1891] values for the absorption coefficients have been used.

Table I. *The Absorption Coefficients of CO_2 (Chr. Bohr and J. C. Bock).*

Temp.	α	Temp.	α
15	1·019	20	0·878
16	0·985	21	0·854
17	0·956	22	0·829
18	0·928	37	0·567
19	0·902	38	0·555

Reduction to 0° C. and 760 mm. Hg has been done in the usual way according to Boyle's and Gay Lussac's laws.

Mass Action Law.

We have now arrived at the point where it will be advisable to discuss the question of the chemical equilibrium which takes place between substances which react with one another in solution.

When the mass action law is expressed as a formula and the apparent activity of the mol B is designated by a_B the following holds good:

The reaction[1] $bB + cC + dD \rightleftarrows mM + nN + oO$ proceeds from left to right with the rate

$$a_B^b \cdot a_C^c \cdot a_D^d \cdot k_{\mathrm{I}}. \quad \text{.....(13)}$$

and from right to left with the rate

$$a_M^m \cdot a_N^n \cdot a_O^o \cdot k_{\mathrm{II}}, \quad \text{.....(14)}$$

therefore

$$a_B^b \cdot a_C^c \cdot a_D^d \cdot k_{\mathrm{I}} = a_M^m \cdot a_N^n \cdot a_O^o \cdot k_{\mathrm{II}} \quad \text{.....(15)}$$

and

$$\frac{a_M^m \cdot a_N^n \cdot a_O^o}{a_B^b \cdot a_C^c \cdot a_D^d} = K. \quad \text{.....(16)}$$

The expression for a reaction like the following (a binary dissociation) is especially simple, $B \rightleftarrows M + N$, namely

$$\frac{a_M \cdot a_N}{a_B} = K. \quad \text{.....(17)}$$

[1] That is to say b mols of B react with c mols of C and d mols of D, giving m mols of $M + n$ mols of $N + o$ mols of O. But the reaction can also be expressed thus—m mols of M react with n mols of N and o mols of O giving b mols of $B + c$ mols of $C + d$ mols of D.

If we wish to express the law for the reacting (solvated) mols in terms of their concentrations we get

$$\frac{C_M \cdot F_{a(M)} \cdot C_N \cdot F_{a(N)}}{C_B \cdot F_{a(B)}} = K, \quad \text{.....(18)}$$

in which the activity of a mol is defined as the concentration multiplied by the apparent activity coefficient. If the apparent activity coefficient is 1, or if

$$\frac{F_{a(M)} \cdot F_{a(N)}}{F_{a(B)}} = 1,$$

(18) reduces to

$$\frac{C_M \cdot C_N}{C_B} = K. \quad \text{.....(19)}$$

When the reaction is in equilibrium and when it is dissociated so much that $xC_{B(T)}$ mols of M and N are formed, (19) then becomes

$$\frac{x^2}{1-x} C_{B(T)} = K, \quad \text{.....(20)}$$

where $C_{B(T)}$ is the concentration B would have if the reaction proceeded completely from right to left.

Osmotic Pressure.

In watery solutions which only contain non-electrolytes the "ideal" conditions for the osmotic pressure are very nearly fulfilled, particularly at higher temperatures, but here also certain corrections must be introduced for hydration (see Findlay [1913]) which will be more pronounced if several solutes are present simultaneously in the solution some of which appropriate considerable quantities of the solvent.

In solutions of electrolytes the conditions will be still more complicated when we consider the osmotic activity of the charged particles (ions). It has until recently been generally accepted, though not without hesitation, that the osmotic activity of the ions was the same function of their concentration as in the case of non-electrolytes, but latterly it has been appreciated that the electrical forces between the ions must diminish their osmotic activity.

Milner (quoted from Bjerrum [1916, 1918, 1919]) has attempted to calculate the magnitude of this effect, which Bjerrum calls the *Milner effect*, without, according to Bjerrum, having actually succeeded in doing so, but as regards the questions dealt with here this is of no importance because we accept the empirical formula given by Bjerrum which will be discussed later on. But first we must recall some of the principal points in Arrhenius' theory of electrolytic dissociation.

Electrolytic Dissociation.

In the following sketch of the fundamental conceptions of the theory of electrolytic dissociation aqueous solutions are exclusively referred to.

Water dissociates to a slight extent into hydrogen and hydroxyl ions,

$$HOH = H^{\cdot} + (OH)'. \quad \text{.....(21)}$$

According to the law of mass action,

$$\frac{a_{H^{\cdot}} \times a_{(OH)'}}{a_{H_2O}} = k. \quad \text{.....(22)}$$

In weak solutions the following equation is very nearly true,

$$a_{H_2O} = K \quad \text{.....(23)}$$

From (22) and (23) we get

$$a_{H^{\cdot}} \times a_{(OH)'} = K_w, \quad \text{.....(24)}$$

where K_w is of the order of 10^{-14}. [See also Lewis, 1916, **1**, p. 325, and S. P. L. Sörensen, 1912, **12**, p. 400.]

When an electrolyte is dissolved in water it will be split up—dissociated—and positively and negatively charged ions will be formed.

The ions carry one, two or more electrostatic units and the dissociation can therefore take place according to the following schemes:

$$\underset{\text{(electrolyte)}}{M_I A_I} \rightleftarrows \underset{\text{(cation)}}{M^{\cdot}_I} + \underset{\text{(anion)}}{A'_I} \quad \text{.....(25)}$$

$$M_I M_I A_{II} \rightleftarrows M^{\cdot}_I + M^{\cdot}_I + A''_{II} \quad \text{.....(26)}$$

$$M_I M_I M_I A_{III} \rightleftarrows M^{\cdot}_I + M^{\cdot}_I + M^{\cdot}_I + A'''_{III} \quad \text{.....(27)}$$

or $$M_I M_I M_I A_{III} \rightleftarrows M^{\cdot}_I + M_I M_I A'_{III} \quad \text{.....(28)}$$

$$M_I M_I A'_{III} \rightleftarrows M^{\cdot}_I + M_I A''_{III} \quad \text{.....(29)}$$

or $$M_{II} A_I A_I \rightleftarrows M_{II} A^{\cdot}_I + A'_I \quad \text{.....(30)}$$

$$M_{II} A^{\cdot}_I \rightleftarrows M^{\cdot\cdot}_{II} + A'_I \quad \text{.....(31)}$$

and so on.

$M^{\cdot}_I$ is a monovalent cation, $M^{\cdot\cdot}_{II}$ a divalent cation, etc.; A'_I and A''_{II} respectively monovalent and divalent anions. There is reason to distinguish between three kinds of electrolytes.

Electrolytes can be divided into:

- Totally dissociated electrolytes
  - Alkali salts and similar salts
  - Strong acids
  - ,, bases
- Electrolytes the dissociation of which varies with the concentration
  - Dissociation varying with the reaction
    - Weak acids
    - ,, bases
    - Ampholytes
  - Complex salts

The acids dissociate according to the following schemes:

For a monovalent acid

$$HA_I \rightleftarrows H^{\cdot} + A'_I, \quad \text{.....(32)}$$

and for a divalent acid

$$H\,H\,A_{II} \rightleftarrows H^{\cdot} + H\,A'_{II} \text{ (partial dissociation)}, \quad \text{.....(33)}$$

$$H\,A'_{II} \rightleftarrows H^{\cdot} + A''_{II}. \quad \text{.....(34)}$$

The bases dissociate as follows:

$$M_{\mathrm{I}}(\mathrm{OH}) \rightleftarrows M'_{\mathrm{I}} + (\mathrm{OH})', \quad \ldots\ldots(35)$$

$$M_{\mathrm{II}}(\mathrm{OH})(\mathrm{OH}) \rightleftarrows M''_{\mathrm{II}} + (\mathrm{OH})' + (\mathrm{OH})'. \quad \ldots\ldots(36)$$

For an ampholyte which can split off one hydrogen ion and one hydroxyl ion per molecule the following hold:

for the hydrogen ion dissociation

$$H\,R(\mathrm{OH}) \rightleftarrows H' + R(\mathrm{OH})', \quad \ldots\ldots(37)$$

for the hydroxyl ion dissociation

$$H\,R(\mathrm{OH}) \rightleftarrows H\,R' + (\mathrm{OH})', \quad \ldots\ldots(38)$$

and for the simultaneous dissociation of H' and $(\mathrm{OH})'$

$$H\,R(\mathrm{OH}) \rightleftarrows H' + (\mathrm{OH})' + 'R'. \quad \ldots\ldots(39)$$

Although the dissociation of weak acids and bases, and ampholytes follows the mass action law, difficulties are encountered with the strong acids and bases which compel us to believe that they are completely dissociated (*i.e.* the reaction progresses completely from left to right), as will now be more fully explained in speaking of salts.

"Strong" Electrolytes.

The question which has caused most trouble in connection with the electrolytic dissociation theory is whether the "strong" electrolytes follow the "mass action law."

We will not discuss further the extremely wide development the subject has undergone; in all the larger textbooks of physical chemistry it is dealt with, and in Arrhenius' [1912] *Theories of Solution* a whole chapter is reserved for it to which the reader is referred. Suffice it to say that in whatever way the dissociation is estimated—whether by depression of the freezing point or by conductivity methods, the mass action law is not fulfilled. In using the method involving the depression of the freezing point which is proportional to the osmotic pressure it was assumed the depression caused by ions and non-electrolytes was the same function of their concentration. As an example of such an estimation the dissociation (25)

$$M_{\mathrm{I}}\,A_{\mathrm{I}} \rightleftarrows M'_{\mathrm{I}} + A'_{\mathrm{I}}$$

may be considered. The solution has a freezing point depression which is i[1] times greater than a non-electrolyte of the concentration of $M_{\mathrm{I}}\,A_{\mathrm{I}}$ (if the reaction proceeded completely from right to left). If we regard the freezing point depression as proportional to the osmolar concentration without corrections for the Milner effect, the following equations will apply:

($C_{M_{\mathrm{I}}A_{\mathrm{I}}(T)}$ is the concentration of the salt when the reaction proceeds completely from right to left.)

$$C_{M'_{\mathrm{I}}} = (i-1)\,C_{M_{\mathrm{I}}A_{\mathrm{I}}(T)} = C_{A'_{\mathrm{I}}} \quad \ldots\ldots(40)$$

and

$$C_{M_{\mathrm{I}}A_{\mathrm{I}}} = (2-i)\,C_{M_{\mathrm{I}}A_{\mathrm{I}}(T)}; \quad \ldots\ldots(41)$$

[1] i is van 't Hoff's coefficient.

substituting in (19) we get

$$\frac{(i-1)^2}{2-i} \cdot C_{M_1A_1(T)} = K. \quad \text{.....(42)}$$

In calculations made from conductivity experiments it was assumed the velocity of the ions was independent of the other ions present in the solution. It was thought therefore that

$$\frac{\text{the equivalent conductivity at the concentration } C_{M_1A_1(T)}}{\text{the equivalent conductivity at infinite dilution}} = \frac{\mu_v}{\mu_\infty} = f_\mu$$

was equal to the value x in (20) and it was subsequently substituted in the equation, but corrections for altered viscosity were often introduced.

As it appeared to be impossible to obtain any concordance between the experiments and the requirements of the mass action law—whether the dissociation was estimated by the methods described or in any other way—either the assumptions which formed the basis of the calculation were erroneous, or it must be concluded the strong electrolytes do not follow the mass action law, a conclusion which under these conditions would be destructive to the law.

It has for many years been known that the dissociation determined by conductivity methods (see for example Jahn [1900]) was not quite accurate while that determined by depression of the freezing point has as a rule been regarded with greater confidence, at any rate by physiologists, although there has been some suspicion about it also.

The problem of elucidating the nature of the complications mentioned seems first to have been mastered by the Dane, Niels Bjerrum [1909, 1916, 1918, 1919].

Dissociation Theory of Bjerrum.

Bjerrum's theory can aptly be called *the theory of the complete dissociation of the strong electrolytes.*

From a number of experiments he carried out in 1906 on the chromium salts Bjerrum came to the conclusion the strong electrolytes were completely dissociated. It was found that they possessed the colour of the ions both in weak and in strong solutions if complex combinations were not formed (Bjerrum)[1]. The fact that the colour of a strong electrolyte was independent of its concentration led him in 1909 to formulate the hypothesis that the strong electrolytes must be taken to be completely dissociated into their ions in solution provided complex compounds were not produced.

In 1919 Bjerrum developed this theory further and showed that a large number of the difficulties of the electrolytic dissociation theory disappeared on his supposition.

It can thus be said that Bjerrum showed in some very characteristic cases

[1] I shall follow extremely closely Bjerrum's exposition which to a large extent is quoted word for word.

that it was not necessary to attribute any catalytic effect to the undissociated acid (in systems where hydrogen ions were the catalyst), which was the case when accounting for the dissociation determined by conductivity.

Bjerrum and Gjaldbaek [1919] succeeded in getting agreement between the calculated and experimental reaction constants for calcium carbonate and their investigations will be briefly discussed later in this work.

They also succeeded in calculating the potential for the concentration cell Hg/HgCl, 0·1*n* KCl/0·01*n* KCl, HgCl/Hg; after the elimination of the diffusion potential the potential was

$$\epsilon = 0{\cdot}0548 \text{ volt.}$$

Reckoned with the Nernst formula

$$\epsilon = 0{\cdot}0591 \log \frac{c_2}{c_1} \text{ volt,} \quad \text{.....(43)}$$

by actual concentration $\epsilon = 0{\cdot}0591$ volt,

(corrected) by the degree of conductivity

$$\epsilon = 0{\cdot}0569 \text{ volt,}$$

(corrected) by the activity coefficient

$$\epsilon = 0{\cdot}0553 \text{ volt.}$$

The following is a translation of a part of Bjerrum's paper in the reports of the Nobel Institute, those sections only being included which are necessary for the understanding of the theory, as it will be indispensable for those wishing to work at the subject to consult the original.

"In the year 1887 Arrhenius enounced his famous hypothesis according to which the ions in a solution of electrolytes are present in the solution in the free state. During the 26 years passed since then this hypothesis has become an indispensable part of physics and chemistry, and even to-day it acts as a fruitful working hypothesis thus showing the powerful richness and force of the original conception. In the course of time, however, several difficulties have attended this hypothesis especially in explaining the properties of strong electrolytes. But after the work of later years these difficulties appear to vanish when considering the effects that must be produced on the properties of the solutions of electrolytes by the electric forces between the ions. The greatness of these effects may be indicated by means of coefficients expressing the relation between the real value of the property in question and the value the property should have held supposing the electric forces did not act between the ions. Thus the activity-coefficient f_a indicates the effect of the interionic forces on the activity of the ions; the conductivity-coefficient f_μ indicates the influence of the interionic forces on the conductivity; the osmotic coefficient f_0 indicates the influence of the interionic forces on the osmotic pressure etc.

If we suppose that the strong electrolytes are completely dissociated into ions we may determine the value of the osmotic coefficient by means of freezing-point determinations. From Noyes's and Falk's excellent exposition

it thus results that the osmotic coefficient of all electrolytes with monovalent ions may, with fair approximation, be rendered by the formula

$$1 - f_0 = k \sqrt[3]{c}, \quad \text{.....(44)}$$

in which k varies between 0·146 and 0·225 the mean being 0·17.

This formula, no doubt, does not agree with Milner's values, but within the limits of concentration where both experiments and Milner's calculation are fairly reliable, viz. $0{\cdot}01m - 0{\cdot}1m$, the agreement is a rather fair one. This fact must be looked on as a support of the assumption that the strong electrolytes are completely dissociated or, at any rate, are much more dissociated than originally assumed.

When the formula of the osmotic coefficient is known, the formula of the activity-coefficient may be deduced by means of the following thermodynamic relation:

$$f_0 + c\frac{\delta f_0}{\delta c} = 1 + c\frac{\delta \ln f_a}{\delta c}. \quad \text{.....(45)}$$

If this be combined with (44) the following expression is obtained:

$$\ln f_a = -4k \sqrt[3]{c}^1. \quad \text{.....(46)}$$

When trying to determine experimentally the value of the activity-coefficient f_a for an ion, various difficulties are thrown in one's way.

First, it is difficult to make sure that the electrolyte is really completely ionised or, if that is not the case, to determine its degree of dissociation. If the molar conductivity of the electrolyte increases with decreasing concentration according to Ostwald-Walden's valence rule it will however be natural, as I have developed formerly, to presume complete dissociation. In many cases this point may be investigated by means of spectro-photometric measurements the colour of the ions being, to a rather high degree, independent of the interionic forces and, on the other hand, greatly changing when the ions combine chemically together.

Secondly, it is not possible, even if the ion concentration is known, to state with certainty what the activity of the ion would have been if the interionic forces did not exist. This difficulty particularly manifests itself in concentrated solutions. As the measure of the activity one should, most likely, take neither the mol number per litre nor the mol number per 1000 g. of water but what is known as the mol fraction,

$$x = \frac{n}{n+n'}, \quad \text{.....(47)}$$

where n is the mol number of the solute and n' the mol number of the solvent. We shall designate this mol fraction as x-concentration. As to the justification for putting the activity equal to the x-concentration we may for instance refer to the excellent exposition of ideal solutions in Alex. Findlay's book: The osmotic pressure.

Thirdly, the water content of the ions in the solution must be considered. When the ions form hydrates in solution the consequence is that the x-con-

[1] Table II gives the corresponding values of the constant factors in equations (44) and (46).

Table II.

$\frac{1-f_0}{\sqrt[3]{c}}$	$\frac{\ln f_a}{2{\cdot}3026\sqrt[3]{c}}$
0·146	0·25
0·17	0·30
0·225	0·39

centrations of the ions will be smaller than they would have been without the hydration. The effect of hydration is well known and frequently discussed. There is, however, another effect of hydration which has not as yet been taken into account; although it is rather of greater importance than the former effect. If we want to determine the activity of an ion by means of potential measurements, say with metal or hydrogen electrodes or with mercurous chloride electrodes, *we obtain for a hydrated ion by means of the ordinary Nernst formula not a measure of the activity of the ion itself but only of the activity of the ion without water. If the ion contains m H_2O we should to obtain the measure of the activity of the ion-hydrate multiply the activity of the ion without water, which we may call A, by the activity of the water to the mth power.* We have a good measure of the activity of the water in the vapour pressure of the solution p divided by the vapour pressure of pure water p_0. So the activity of the actual ion may be put at

$$a = A\left(\frac{p}{p_0}\right)^m. \quad \text{.....(48)}$$

Strange to say no account has till now been taken of this correction in spite of the fact that in the electrometric determination of the hydrogen-ion-concentration, so frequently used of late, it is of considerable importance, the hydrogen-ion being very strongly hydrated. The measurement of the potential of a hydrogen-electrode in a solution does not directly give us either the hydrogen-ion-concentration or the hydrogen-ion-activity, but only a calculative quantity which may be designated as the activity of the dehydrated hydrogen-ion. It is only for want of due consideration of this fact that the conclusion that the hydrogen-ion-activity increases by plentiful addition of neutral salts to hydrochloric and other strong acids has been arrived at (comp. for instance Harned, *Journ. Amer. Chem. Soc.* **37**, 2460 (1915), **38**, 1986 (1916)). This correction should also be made in the case of activity determinations founded on the solubility of salts.

Fourthly, the exact determination of ion-activities in concentrated solutions is rendered difficult by the association of the water, which is of importance in calculating the x-concentration, and which is not properly known."

By making calculations based on the above Bjerrum has succeeded in estimating the amount of hydrate water of the ions.

F_a is the apparent activity coefficient and f_a the true activity coefficient. $-\log F_a$ is the apparent activity exponent.

In Fig. 1 which is reproduced here Bjerrum shows the relation existing between F_a and c for different degrees of hydration of the ions.

While it is the hydrogen ion concentration, as Bjerrum [1916, 1918] has shown for the catalytic action of hydrogen ions (esterification of organic acids), which is the determining factor, it is the apparent hydrogen ion activity which is the important factor in the mass action law relating to those chemical actions in which the hydrogen ions take part. The apparent hydrogen ion activity can be determined by the electrical method in the same way as the "hydrogen ion concentration" has hitherto been estimated.

In measuring the potential difference between a hydrogen-platinum elec-

[1] It may be noted that Bjerrum is using the symbols a and A with a significance differing from that which we have accepted in these articles.

trode and a solution, and a constant electrode we have the following form of Nernst's equation,

$$p_{H^+} = -\log a_{H^+} = \frac{E - E_0}{0{\cdot}0577 + 0{\cdot}0002\,(t - 18°)}, \quad \ldots\ldots(49)$$

E being the measured potential and E_0 a constant dependent upon the electrode used for comparison. If a $0{\cdot}1n$ KCl—HgCl electrode ($0{\cdot}1n$ calomel electrode) is used, S. P. L. Sörensen on the basis of conductivity experiments at 18° obtained

$$E_0 = 0{\cdot}3377 \text{ volt (Sörensen).} \quad \ldots\ldots(50)$$

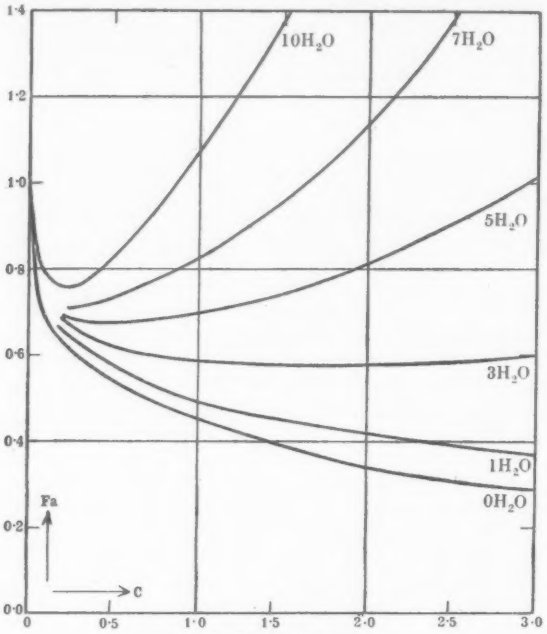


Fig. 1. (After Bjerrum.)

Bjerrum and Gjaldbaek [1919] from calculations based on the activity coefficient obtained

$$E_0 = 0{\cdot}3348 \text{ (Bjerrum)} \quad \ldots\ldots(51)$$

from which

$$E_0 \text{ (Sörensen)} - 0{\cdot}0029 = E_0 \text{ (Bjerrum)}^1. \quad \ldots\ldots(52)$$

Both results are valid for a hydrogen pressure of $760 - f$ mm. Hg where f is the pressure of saturated aqueous vapour at the temperature in question. If the hydrogen pressure is different, *e.g.* P_{H_2}, the following value (e) must be added to E,

$$e = \frac{0{\cdot}0001983}{2}\,(273 + t°)\log\frac{P_{H_2}}{760 - f}, \quad \ldots\ldots(53)$$

where $t°$ is the temperature on the centigrade scale, P_{H_2} is the H_2 pressure in the electrode, and f is the pressure of saturated aqueous vapour at $t°$.

[1] At 38° E_0 (Bjerrum) = 0·3313.

It will be observed that Bjerrum's E_0 is 2·9 millivolts less than Sörensen's. The corresponding difference in $p_{H^\cdot}$ from 5·00 to 8·00 at room temperature or body temperature can with sufficient accuracy be expressed by

$$p_{H^\cdot}\text{ (Bjerrum)} = p_{H^\cdot}\text{ (Sörensen)} + 0{\cdot}048. \quad \text{.....(54)}$$

It is thus easy to transfer the values from one scale to another.

As will be seen from Bjerrum's reasoning,

$$F_a\text{ (Ion)}\ C_{\text{Ion}} = a_{\text{Ion}}. \quad \text{.....(55)}$$

In weak solutions,

$$\log f_a\text{ (Ion)} = \log F_a\text{ (Ion)} \quad \text{.....(56)}$$

and the concentration of an ion can be calculated when its activity is known.

For hydrogen ions according to Bjerrum [1916, 1918] the following equation holds,

$$-\log f_a\text{ (H)}^\cdot = 0{\cdot}2\sqrt[3]{c}. \quad \text{.....(57)}$$

For the bicarbonate ion k is not known but it will be shown later that k for sodium bicarbonate is about 0·46.

For an n-valent ion, according to Bjerrum and Gjaldbaek [1919, p. 79],

$$-\log f_a = nk\sqrt[3]{c}, \quad \text{.....(58)}$$

where k has the same value as for a monovalent ion in the same solution, and where it varies within the same limits as in the case of monovalent ions. Table III, according to Bjerrum, gives the values of the deviation coefficients for KCl.

Table III.

Molec. conc.	f_0	f_a	$f_\mu = \frac{\mu_v}{\mu_\infty}$
0·001	0·985	0·943	0·979
0·01	0·963	0·882	0·941
0·1	0·932	0·762	0·861
1·0	0·854	0·552	0·755

Since $f_a(\text{KCl}) = \sqrt{f_a(\text{Cl}')f_a(\text{K}^\cdot)}$.

It is a matter of fact that all salts do not follow the same law of dissociation. Thus while the salts of the alkali metals and presumably also the majority of those of the alkaline earths are completely dissociated (Bjerrum and Gjaldbaek [1919], Gjaldbaek [1921]), this is not the case for many of the salts of the heavy metals. The latter form complex compounds, that is to say they are only partially dissociated—often only very slightly dissociated. The equilibrium between the ions and the undissociated salts is determined in a similar manner to that between hydrogen or hydroxyl ions and the electrolytes the dissociation of which varies with the reaction, and the remarks anent these can almost be directly applied to the complex salts (electrolytes the dissociation of which varies with the concentration) by substituting metal ions for hydrogen ions.

As it is by no means a matter of course that a salt is completely dissociated it will be one of the objects of the present work to investigate whether protein salts are complex or not. The working hypothesis that alkali albuminates and

protein salts with Cl′ and HCO'_3 are completely dissociated has been adopted throughout, and it has been investigated whether any facts following from this contradicted the hypothesis.

Complications arising from Heterogeneity.

The following equation holds for a heterogeneous solution,

$$\frac{C_{S_y}(100-Q)}{100} + \frac{C_{S_y}DQ}{100} = C_{S_t}, \quad \text{.....(59)}$$

where Q is the volume of the internal phase expressed as a percentage of the total volume of the solution; C_{S_y} is the true concentration of the solute in the external phase, the possibility of an aggregation being neglected, and C_{S_i} is the concentration in the inner phase; D is a proportionality factor determined by

$$D = \frac{C_{S_i}}{C_{S_y}}, \quad \text{.....(60)}$$

and C_{S_t} is the concentration of the solute in the whole system provided it is evenly distributed over the whole volume. We shall call C_{S_t} the *mean concentration*.

In biological chemistry only scant attention has been paid to the conditions expressed by (59) but there are a number of investigators who have been cognizant of them. Thus Hamburger [1902] was the first to recognise the importance of the volume of the disperse phase in experiments on the osmotic conditions of the blood corpuscles.

Rona [1910, 1913, 1, 2] and his collaborators also corrected for the volume of the disperse phase in dialysis of serum but they did not discuss any more than Hamburger the equation of the solubility of the solute in the continuous phase.

S. P. L. Sörensen in his monumental work *Studies on Proteins* was the first thoroughly to appreciate these conditions and the author of this essay willingly admits he has been greatly influenced by the above work.

After the appearance of *Studies on Proteins* Rich. Ege [1920, 1, 2, 3, 4; 1921, 1, 2, 3, 4] published an excellent piece of work on the partition of glucose between the blood cells and serum in which he pays the proper attention to the characteristic qualities of colloid solutions just mentioned.

In Henderson's and Hasselbalch's equations the heterogeneity of the solution is not allowed for and it was this that originally prompted me to investigate the theory of these equations.

T. R. Parsons in 1917 called attention to the difficulties caused by the different distribution of bicarbonate between the blood corpuscles and serum. L. S. Fridericia [1920] as well as Joffe and Poulton [1920] have recently determined the conditions of distribution but none of these authors regarded the serum and the fluids of the blood corpuscles as heterogeneous systems separate from one another. The work of all these authors is extremely valuable in relation to the questions before us and it will be fully dealt with later in this work.

CHAPTER II

THE HYDRATION OF CARBON DIOXIDE AND THE DISSOCIATION OF CARBONIC ACID.

The Derivation of Henderson's and Hasselbalch's Equations and the Modification of these in accordance with Bjerrum's Theory. The Limits within which the Derived Equations are Valid.

In 1 cc. of water in equilibrium with a gas mixture in which the tension of CO_2 is P_{CO_2}mm. Hg, there will be dissolved according to Henry's law (equation (1)),

$$a = \frac{P_{CO_2}}{760}\alpha \text{ cc. } CO_2 \text{ (at 0° and 760 mm. Hg).}$$

Some of the dissolved carbon dioxide is hydrated, forming carbonic acid,

$$CO_2 + H_2O \rightleftarrows H_2CO_3, \quad \text{.....(61)}$$

and the mass action law gives for this reaction

$$\frac{A_{CO_2}\, a_{H_2O}}{a_{H_2CO_3}} = k_0. \quad \text{.....(62)}$$

Since a_{H_2O} can be regarded as constant under the experimental conditions,

$$\frac{a_{H_2CO_3}}{A_{CO_2}} = K_0, \quad \text{.....(63)}$$

and therefore from (7) and (11),

$$a_{H_2CO_3} = P_{CO_2}\, \alpha K_0\, 0{\cdot}00005910. \quad \text{.....(64)}$$

This equation in accordance with what was said on p. 157 applies to watery solutions also, with the proviso that K_0 is the same for pure water and for watery solutions. How far this is the case cannot be estimated at present, but as from (62) and (63)

$$K_0 = \frac{a_{H_2O}}{k_0} \quad \text{.....(65)}$$

and we regard a_{H_2O} as constant, K_0 will only vary from one solution to another if k_0 varies.

Such a variation under the conditions investigated is improbable, but if it should happen it would be without importance for the following argument and would be included in the total constants. Only in the investigation of the extent to which the activity of the bicarbonate ion conforms to Bjerrum's [1916, 1918, 1919] equation for the calculation of the activity coefficient, would such a condition be of real importance and invalidate the proof. Assuming however that Bjerrum's theory is correct the above-mentioned investigation can be used as a proof that K_0 does not vary appreciably with the ionic concentration within the limits of concentration investigated.

In equation (63)

$$\frac{a_{H_2CO_3}}{A_{CO_2}} = K_0,$$

$a_{H_2CO_3}$ is the apparent activity of carbonic acid, and in so far as it is not

hydrated (*i.e.* it is not H_2CO_3, H_2O for example), we have $a_{H_2CO_3} = A_{H_2CO_3}$. The error involved by putting

$$\frac{a_{H_2CO_3}}{A_{CO_2}} = \frac{C_{H_2CO_3}}{C_{CO_2}} \quad \text{.....(66)}$$

will in all cases be small, as the activity coefficient of non-electrolytes is an expression of the salting out effect which ought to be about the same for all non-electrolytes. K_0 indicates therefore how much of the dissolved CO_2 is hydrated. Experiment has shown that K_0 is small. Thus Thiel and Strohecker [1914] have calculated from the rapidity with which CO_2 neutralises bases that only $\frac{1}{2}$ % of the dissolved CO_2 is hydrated and therefore our K_0 is roughly 0·005.

Walker and Cormack [1900] make some remarks in their experiments on conductivity in CO_2 solutions about the value of K_0. They show that such solutions of carbonic acid fulfil the requirements of the mass action law when the total amount of dissolved CO_2 is substituted for the carbonic acid in the equation. If only a small part of the dissolved CO_2 was hydrated the mass action law would not be satisfied in the form employed and they think therefore they are right in concluding about half the dissolved CO_2 is hydrated (which means that our K_0 should be 0·5). Walker and Cormack's determinations however certainly do not justify them in drawing these conclusions.

Carbonic acid dissociates in the following manner:

$$\underset{\text{(Carbonic acid)}}{H_2CO_3} \rightleftarrows \underset{\text{(Hydrogen ion)}}{H^{\cdot}} + \underset{\text{(Bicarbonate ion)}}{HCO'_3} \quad \text{(1st dissociation), ...(67)}$$

$$\underset{\text{(Bicarbonate ion)}}{HCO'_3} \rightleftarrows \underset{\text{(Hydrogen ion)}}{H^{\cdot}} + \underset{\text{(Monocarbonate ion)}}{CO''_3} \quad \text{(2nd dissociation). .....(68)}$$

From the mass action law

$$\frac{a_{H^{\cdot}} a_{HCO'_3}}{a_{H_2CO_3}} = k_1 \text{ (1st dissociation of carbonic acid), ...(69)}$$

$$\frac{a_{H^{\cdot}} a_{CO''_3}}{a_{HCO'_3}} = K_2 \text{ (2nd dissociation of carbonic acid). .....(70)}$$

Multiplying equations (63) and (69) we get

$$\frac{a_{H^{\cdot}} a_{HCO'_3}}{A_{CO_2}} = k_1 K_0, \quad \text{.....(71)}$$

and therefore

$$\frac{a_{H^{\cdot}} a_{HCO'_3}}{A_{CO_2}} = K_1. \quad \text{.....(72)}$$

Since, almost without exception before the appearance of Bjerrum's theory, we put

$$a_{H^{\cdot}} = C_{H^{\cdot}}, \quad \text{.....(73)}$$

$$a_{CO_2} = C_{CO_2}, \quad \text{.....(74)}$$

$$a_{HCO'_3} = C_{HCO'_3}\gamma = (C_{HCO'_3} f_\mu), \quad \text{.....(75)}$$

where γ was the "conductivity dissociation" of the bicarbonate in the concentration under investigation, (72) became

$$\frac{C_{H^{\cdot}} C_{HCO'_3}\gamma}{C_{CO_2}} = K_1, \quad \text{.....(76)}$$

which was transformed into

$$C_{H^{\cdot}} = \frac{K_1}{\gamma} \frac{C_{CO_2}}{C_{HCO'_3}}. \quad \text{.....(77)}$$

This is Henderson's equation.

Equations like (76) and (77) have repeatedly been used in physical chemistry, for instance in W. Nernst's experiments on the dissociation constants of weak monovalent acids (cited from Datta and Dhar [1915]), in Datta and Dhar's [1915] determinations of the second dissociation constant of weak divalent acids, and in investigations on the second dissociation constant of carbonic acid by McCoy and Smith [1911] and McCoy and Test [1911], Auerbach and Pick [1912], and Seyler and Lloyd [1917, 1, 2], and lastly in work upon the solubility of the carbonates of the alkaline earths, for example, by Johnston [1916].

An equation analogous to (72) is one derived by Bjerrum and Gjaldbaek [1919, p. 82, equation (24)].

In logarithmic form (77) becomes

$$p_{H^\cdot} = pK_1 + \log C_{HCO'_3} - \log C_{CO_2} + \log \gamma. \quad \text{.....(78)}$$

As will be shown later in considering the limits between which an equation similar to (77) is valid, $C_{HCO'_3}$ in the cases treated by Hasselbalch and Henderson (as always in these investigations) can be assumed to have originated from the combined carbonic acid, as the amount of HCO'_3 derived from the dissociation of the dissolved carbonic acid is negligible in comparison with it. If we know the total quantity of CO_2 in a solution and its pressure in a gaseous phase in equilibrium with the solution, the temperature and the relative absorption coefficient of the solution, it is easy to calculate the quantity of combined carbonic acid by subtracting the amount dissolved from the total amount. *If now it is assumed all the combined carbonic acid is in the form of bicarbonate*, and the CO_2 is expressed in volumes per cent., (77) compared with (5) becomes

$$C_{H^\cdot} = \frac{K_1 \text{ vol. \% dissolved } CO_2\ 0{\cdot}0004492}{\gamma \text{ vol. \% combined } CO_2\ 0{\cdot}0004492}, \quad \text{.....(79)}$$

$$C_{H^\cdot} = \frac{K_1 \text{ vol. \% dissolved } CO_2}{\gamma \text{ vol. \% combined } CO_2}. \quad \text{.....(80)}$$

This is Henderson's equation in a simplified form, expressed in the same way as that of Hasselbalch.

Hasselbalch [1916, 2] regarded carbonic acid as a divalent acid and put the concentrations in (77) in terms of normality, assuming a solution which contained a gram-molecule of CO_2 in a litre to be twice normal as regards carbonic acid, while he regarded a solution which contained a gram-molecule of CO_2 bound as bicarbonate in a litre, as normal with respect to bicarbonate[1].

Equation (77) under these circumstances becomes

$$C_{H^\cdot} = \frac{K_1}{\gamma} \frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2} 2. \quad \text{.....(81)}$$

And since Hasselbalch substitutes K_1 for $\frac{K_1}{\gamma}$ his equation becomes

$$C_{H^\cdot} = K_1 \frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2} 2. \quad \text{.....(82)}$$

[1] Parsons [1919] and L. Michaelis [1920] have recently pointed out that Hasselbalch uses this unusual convention and Michaelis has protested against it.

If we now compare (80) with (82) it will be seen that

$$\frac{K_1\,(\text{Henderson})}{\gamma} = 2K_I\ (\text{Hasselbalch}). \quad \ldots\ldots\ldots\ldots\ldots\ldots(83)$$

Hasselbalch's equation in logarithmic form becomes

$p_{H^\cdot} = p_{K_1} + \log$ vol. % combined $CO_2 - \log$ vol. % dissolved $CO_2 - 0{\cdot}3010$,

and when (83) is put in logarithmic form we get

$$pK_1\ (\text{Henderson}) + \log\gamma = pK_I\ (\text{Hasselbalch}) - 0{\cdot}3010. \ldots\ldots(84)$$

Although the above appears to be correct it should be noted that Hasselbalch *determined his total constant* by the potentiometrical method. If this is recognised (83) becomes

$$\frac{K_1\,(\text{Henderson})}{F_a(HCO'_3)} = 2K_I\ (\text{Hasselbalch}) \quad \ldots\ldots\ldots\ldots\ldots\ldots(85)$$

and (84) is transformed accordingly, but at the same time a correction ought to be introduced into all Hasselbalch's calculations so that p_{K_I} always becomes 0·048 times larger (given by the difference between Sörensen's and Bjerrum's E_0).

If we abide by what is the usual custom in the literature of physical chemistry there is no doubt we ought to use molar concentration and not normality in such equations, and as a similar convention also has priority in physiological literature, Hasselbalch's mode of expression should be given up.

As the assumptions underlying (73), (74) and (75) are no longer tenable the equation should be modified in accordance with Bjerrum's requirements. When the mols in the equation with the exception of $a_{H^\cdot}$ are expressed by their concentrations, (72) becomes

$$\frac{a_{H^\cdot} C_{HCO'_3} F_a(HCO'_3)}{C_{CO_2} F_a(CO_2)} = K_1. \quad \ldots\ldots\ldots\ldots\ldots\ldots(86)$$

It will be seen from (54) that

$$1{\cdot}117\ a_{H^\cdot} = C_{H^\cdot}\ (\text{Sörensen}), \quad \ldots\ldots\ldots\ldots\ldots\ldots(87)$$

and we thus obtain

$$\frac{C_{H^\cdot}(\text{Sörensen})\, C_{HCO'_3}}{C_{CO_2}} = K_1 \frac{F_a(CO_2)\ 1{\cdot}117}{F_a(HCO'_3)}. \quad \ldots\ldots\ldots\ldots\ldots\ldots(88)$$

Substituting

$$K'_1 = \frac{K_1}{F_a(HCO'_3)} \quad \ldots\ldots\ldots\ldots\ldots\ldots(89)$$

we get from (88) and (85)

$$K'_1 = \frac{1{\cdot}793\ K_1\ (\text{Hasselbalch})}{F_a(CO_2)}, \quad \ldots\ldots\ldots\ldots\ldots\ldots(90)$$

and from (83) and (90)

$$K'_1 = \frac{0{\cdot}896\ K_1\ (\text{Henderson})}{\gamma\, F_a(CO_2)}. \quad \ldots\ldots\ldots\ldots\ldots\ldots(91)$$

If the relative absorption coefficient for blood is taken as 0·92 (Bohr [1905]), $F_a(CO_2)$ becomes 1·087 and we get for blood

$$\text{Blood} \begin{cases} K'_1 = 1{\cdot}65\ K_I\ (\text{Hasselbalch}), & \ldots\ldots\ldots\ldots\ldots\ldots(92) \\ pK'_1 = pK_I\ (\text{Hasselbalch}) - 0{\cdot}218. & \ldots\ldots\ldots\ldots\ldots\ldots(93) \end{cases}$$

Now that we have developed the relations between the various constants

we will revert to (72) and express it in the same way as Hasselbalch's equation by using (89)

$$a_{H^{\cdot}} = K'_1 F_a (CO_2) \frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2} \quad \text{.....(94)}$$

or

$$a_{H^{\cdot}} = K'_1 \frac{P_{CO_2 a}}{7{\cdot}60 \text{ vol. \% combined } CO_2}. \quad \text{.....(95)}$$

Putting (94) in logarithmic form we get

$$p_{H^{\cdot}} \text{ (Bjerrum)} = pK'_1 + \log \text{vol. \% combined } CO_2 - \log \text{vol. \% dissolved } CO_2 - \log F_a (CO_2). \quad \text{.....(96)}$$

We have now worked out the relations between Henderson's, Hasselbalch's and our own equations as expressed in (90) and (91), but a considerable qualification of their significance must be made.

Henderson's and Hasselbalch's equations were evolved, as will be remembered, on the assumption that the degree of dissociation measured by conductivity gave the degree of activity of the bicarbonate, but since this is incorrect as Bjerrum later has shown, (73), (74), (75), (76), (77), (78), (79), (80), (81), (82), (83), (84), and (91) are only approximations to the true equations and have *only mathematical significance*, while (88) and (90) are correct actually as well as mathematically.

It is perhaps also worth pointing out that (90) only holds good if K_I (Hasselbalch) and K'_1 are calculated from the same experiments or from experiments which give the same results with the same method of calculation, and that Sörensen's E_0 (50) should be used for the calculation of K_I (Hasselbalch), while E_0 (Bjerrum) (51) should be used for the calculation of K'_1. If (90) is not satisfied by a correct calculation from two series of experiments it is proof that the experimental results do not agree.

In the equations evolved up to the present we have assumed without hesitation that all the combined carbonic acid is present as bicarbonate. *We will now inquire how far the equations are valid if the second dissociation of carbonic acid* (68) *takes place to any considerable extent.* We will first find out, however, the reaction at which this happens.

Equation (70)

$$\frac{a_{H^{\cdot}} a_{CO''_3}}{a_{HCO'_3}} = K_2$$

can be transformed into

$$\frac{C_{CO''_3}}{C_{HCO'_3}} = \frac{K_2}{a_{H^{\cdot}}} \frac{F_a (HCO'_3)}{F_a (CO''_3)}. \quad \text{.....(97)}$$

The value of the right side of the equation is still not accurately known in spite of a great deal of work on the subject, since McCoy's [1911], Auerbach and Pick's [1912], Shield's [1893], and Seyler and Lloyd's [1917, 1] results must be recalculated according to the new ideas. Bjerrum and Gjaldbaek [1919] give $K_2 = 10^{-10{\cdot}22}$ from which we may expect to get a good approximation by putting

$$K_2 \frac{F_a (HCO'_3)}{F_a (CO''_3)} = 10^{-10} = K'_2. \quad \text{.....(98)}$$

It should however be noted that the value may vary somewhat with the ionic concentration.

If with this value $\frac{C_{CO''_3}}{C_{HCO'_3}}$ is calculated from (97) the table below is obtained.

Table IV.

$a_{H^\cdot}$	$p_{H^\cdot}$	$\frac{C_{CO''_3}}{C_{HCO'_3}}$ room temp.	$a_{H^\cdot}$	$p_{H^\cdot}$	$\frac{C_{CO''_3}}{C_{HCO'_3}}$ room temp.
1×10^{-7}	7·00	0·001	$0{\cdot}25 \times 10^{-8}$	8·60	0·040
0·5	7·30	0·002	0·2	8·70	0·050
0·3	7·52	0·003	0·17	8·77	0·059
0·2	7·70	0·005	0·15	8·82	0·067
0·15	7·82	0·007	0·13	8·89	0·077
1×10^{-8}	8·00	0·010	0·11	8·96	0·091
0·5	8·30	0·020	1×10^{-9}	9·00	0·100
0·3	8·52	0·033			

It will be seen from the table that at $p_{H^\cdot}$ 7·00 the ratio is only $\frac{1}{1000}$, at $p_{H^\cdot}$ 8·00, $\frac{1}{100}$ and at 9·00 it is $\frac{1}{10}$. It may be concluded from this that in reactions of physiological importance all the carbonate may be considered to be in the form of bicarbonate—a result which numerous workers have previously arrived at in a similar way (*e.g.* L. Meyer [1857], Heidenhain and L. Meyer [1863], Zuntz [1868], Chr. Bohr [1909], van Slyke and Cullen [1917], Parsons [1919], Bayliss [1915] and others). We shall revert briefly to this subject in discussing the variations of volume of the blood corpuscles.

It will now be shown that with a little alteration the equations evolved are valid even if the reaction is so alkaline that a large amount of monocarbonate ions are present in the solution. Instead of the concentration, the corresponding amounts of CO_2 are substituted expressed in volumes per cent., and the amount

corresponding to C_{CO_2} is called S_0,
,, $C_{HCO'_3}$,, S_1,
,, $C_{CO''_3}$,, S_2.

(94) then becomes $$a_{H^\cdot} = K'_1 F_a (CO_2) \frac{S_0}{S_1}. \quad \text{.....(99)}$$

And (97) combined with (98)

$$a_{H^\cdot} = K'_2 \frac{S_1}{S_2}. \quad \text{.....(100)}$$

Calling the total amount of combined CO_2, B, we get

$$S_1 + S_2 = B, \quad \text{.....(101)}$$

and from (100) and (101) $$S_1 = \frac{a_{H^\cdot} B}{K'_2 + a_{H^\cdot}}. \quad \text{.....(102)}$$

When this is substituted in (99) we get a quadratic equation

$$a_{H^\cdot} = \frac{S_0 K'_1 F_a (CO_2)}{2B} \pm \sqrt{\frac{(S_0 K'_1 F_a (CO_2))^2 + 4 S_0 K'_1 F_a (CO_2)^2 K'_2 B}{4B^2}}.$$

The numerator under the square root sign is equal to

$$(S_0 K'_1 F_a (CO_2) + 2K'_2 B)^2 - 4K'^2_2 B^2.$$

$4K'^2_2 B^2$ can be neglected as K'^2_2 is very small, and as the negative sign is without physical significance, we get

$$a_{H^\cdot} = \frac{K'_1 F_a (CO_2) S_0}{B} + K'_2. \quad \text{.....(103)}$$

The equation (103) *thus developed is valid generally* (independent of hydrolysis of the ions), *so long as the concentration of the combined carbonic acid is large compared with the concentration of the dissolved* CO_2, *and so long as all the combined carbonic acid is present as monocarbonate and bicarbonate.*

It will be noticed that it is identical with the earlier equation (94) (the modified Henderson-Hasselbalch equation) when K'_2 is small compared with

$$K'_1 F_a\,(CO_2)\,\frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2}.$$

In the following short table the first column contains values of

$$K'_1 F_a\,(CO_2)\,\frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2};$$

the second column contains the corresponding values of

$$K'_1 F_a\,(CO_2)\,\frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2} + K'_2,$$

K'_2 being again 10^{-10}; in the last column the corresponding $p_{H^\cdot}$ values are given.

Table V.

$K'_1 F_a\,(CO_2)\,\frac{S_0}{S_1}$	$K'_1 F_a\,(CO_2)\,\frac{S_0}{S_1} + K'_2$	$p_{H^\cdot}$
10^{-8}	$1{\cdot}010 \times 10^{-8}$	7·996
10^{-9}	$1{\cdot}100 \times 10^{-9}$	8·959
10^{-10}	$2{\cdot}000 \times 10^{-10}$	9·699

It will be seen from the table *that at reactions more acid than* 10^{-8} *the correction is negligible and the equation becomes identical with the modified Henderson-Hasselbalch equation.*

We have still to investigate how small the quantity of combined carbonic acid can be without being small in comparison with the amount of bicarbonate arising from the dissolved CO_2.

The hydrogen ions in a solution containing dissolved CO_2 may either

(1) exclusively be derived from the dissociated carbonic acid (except for the minimal amount due to the dissociation of water), or

(2) partly come from the carbonic acid and partly from another acid, or

(3) the reaction may be so acid that the carbonic acid is undissociated, in which case there can be no carbonate present in the solution and the equation does not apply.

We will only consider the case when carbonic acid is the sole source of hydrogen ions, because the field is more restricted than in the other cases.

Let us call the concentration of bicarbonate ions which is equivalent to the amount of available base, C_m, the bicarbonate ions which are derived from the dissociation of carbonic acid being, as already mentioned, equal to $C_{H^\cdot}$. Equation (86) taken in conjunction with (89) and (85) then gives

$$\frac{C_{H^\cdot} F_a(H^\cdot)\,(C_m + C_{H^\cdot})}{C_{CO_2}} = K'_1 F_a\,(CO_2), \quad \ldots\ldots\ldots\ldots\ldots\ldots (104)$$

from which

$$C_{H^\cdot} = -\frac{C_m}{2} \pm \sqrt{\frac{C_m^2}{4} + \frac{K'_1 F_a\,(CO_2)\,C_{CO_2}}{F_a(H^\cdot)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (105)$$

in which the negative sign is without actual meaning. If C_m is equal to 0, (105) reduces to

$$a_{H^\cdot} = \sqrt{K'_1 F_a(CO_2) F_a(H^\cdot) C_{CO_2}}. \quad \text{.....(106)}$$

(106) applies to reactions in solutions of CO_2 where there is no combined carbonic acid.

A hard and fast rule for using (105) instead of the modified Henderson-Hasselbalch equation is difficult to give, but a good approximate rule may be arrived at in the following way.

(104) is compared with (86) and (89) in the form

$$\frac{C_{H^\cdot} F_a(H^\cdot) C_m}{C_{CO_2}} = K'_1 F_a(CO_2). \quad \text{.....(107)}$$

The difference between (104) and (107) is due to the difference between the factors C_m and $(C_{H^\cdot} + C_m)$. If $C_{H^\cdot}$ is small in comparison with C_m, the equations become the same. Making the approximate assumption that $C_{H^\cdot}$ is equal to $a_{H^\cdot}$ we will evaluate $\frac{C_{H^\cdot}}{C_m}$. $a_{H^\cdot}$ is calculated from (94) which only differs from (105) in form, and an estimation of the value of $\frac{a_{H^\cdot}}{C_m}$ is made. If this does not exceed 0·01 the use of equation (94) is admissible.

Table VI.

$a_{H^\cdot}$	C_m	Vol. % combined CO_2	mm. Hg, CO_2
10^{-6}	10^{-4}	0·22	0·563
10^{-5}	10^{-3}	2·24	53·2
10^{-4}	10^{-2}	22·39	4710

In the table the values which C_m must have at different reactions so that the above requirements for the applicability of (94) may be fulfilled, are given. In the third column C_m is expressed in volumes per cent. of CO_2 and in the fourth column the corresponding tension of CO_2 calculated with the help of the constants determined for sodium bicarbonate in Chapter VIII.

Résumé.

From the above results it may be said that in homogeneous solutions:

I. Henderson's equation is in agreement with the equation evolved on the basis of Bjerrum's new dissociation theory when $K_1 \frac{F_a(CO_2)}{F_a(HCO'_3)}$ is substituted for $\frac{K_1}{\gamma}$ in the former.

II. Hasselbalch's $K_I = K_1 \frac{F_a(CO_2) \cdot 0{\cdot}557}{F_a(HCO'_3)}$.

III. The value found then becomes $a_{H^\cdot}$ (Bjerrum), not $C_{H^\cdot}$ (Sörensen), and instead of $p_{H^\cdot}$ (Sörensen) we have $p_{H^\cdot}$ (Bjerrum).

IV. The limits for the modified Henderson-Hasselbalch equation are given.

V. The complete equations for the relation between the hydrogen ion activity, the amount of dissolved CO_2 and the combined carbonic acid are evolved.

CHAPTER III

THE DEVELOPMENT OF A MODIFIED HENDERSON-HASSELBALCH EQUATION FOR USE IN THE CASE OF HETEROGENEOUS SOLUTIONS.

As already hinted at several times the conditions in heterogeneous solutions are complicated by the fact that the concentration of the solute in the individual phases is not directly given by its mean concentration, as the solute cannot be assumed to be evenly distributed over the whole system. Further we cannot regard the activity coefficients in the single phases as already known, and lastly there is the possibility that the solute may be concentrated by adsorption in the interfaces between the different phases.

The influence of the first condition on the determination of reaction by Henderson's and Hasselbalch's equations has latterly been recognised by Fridericia [1920], Parsons [1917] and by Joffe and Poulton [1920], but they have not realised the full significance of it, only regarding serum-blood cells as a heterogeneous system, and failing to appreciate that serum (plasma) and the fluid of the blood cells are likewise heterogeneous systems.

Fridericia's and Parsons' contributions appeared while the experimental part of this research was in progress, but Joffe and Poulton's work only came to my notice after I had embarked upon the literary section. These investigations are of the greatest importance for the questions here considered and they will be fully dealt with in a later chapter of this work.

We will proceed with our calculations *under the assumption* that all the combined carbonic acid is present as bicarbonate (that no CO_2 is bound by complex combinations or adsorbed), and we will investigate how the experimental results accord with this assumption.

Let Vol. % combined carbonic acid $= \beta$,

β being the mean concentration in the heterogeneous solution. Let us first consider a system of two watery phases in mutual equilibrium, and in equilibrium with a gaseous phase containing CO_2.

100 parts of the system consist of q parts of phase (2) and $100 - q$ parts of phase (1).

From this we get

$$100 \times \frac{\text{vol. of phase (2)}}{\text{vol. of phase (1)} + \text{vol. of phase (2)}} = q. \quad \text{.....(108)}$$

Also

$$\frac{\text{Vol. \% combined } CO_2 \text{ in phase (2)}}{\text{Vol. \% combined } CO_2 \text{ in phase (1)}} = d. \quad \text{.....(109)}$$

Seeking now the amount of combined CO_2 in the single phases expressed by β, d and q,

$$\frac{V_{(1)}qd}{100} + \frac{V_{(1)}(100-q)}{100} = \beta, \quad \text{.....(110)}$$

$$V_{(1)} = \frac{100\beta}{100 - q(1-d)} \text{ (vol. \% combined } CO_2 \text{ in phase (1))}, \quad \text{...(111)}$$

and

$$V_{(2)} = \frac{100d\beta}{100 - q(1-d)} \text{ (vol. \% combined } CO_2 \text{ in phase 2))}. \quad \text{...(112)}$$

From (111) in conjunction with (95) we get

$$a_{H'(1)} = K'_{1(1)} \frac{100 - q(1-d)}{100} \times \frac{P_{CO_2}\alpha}{7{\cdot}60\beta}, \quad \ldots\ldots\ldots\ldots\ldots\ldots(113)$$

where $a_{H'(1)}$ is the apparent hydrogen ion activity in phase (1) and $K'_{1(1)}$ is K_1 divided by the apparent activity coefficient of HCO'_3 in phase (1).

Analogous to this we have

$$a_{H'(2)} = K'_{1(2)} \frac{100 - q(1-d)}{100d} \times \frac{P_{CO_2}\alpha}{7{\cdot}60\beta}. \quad \ldots\ldots\ldots\ldots\ldots\ldots(114)$$

$K'_{1(1)}$ and $K'_{1(2)}$ are not different from K'_1 in a homogeneous solution and the indices only signify that $F_a(HCO'_3)$ refers to a particular solution.

Comparing (95) with (113) and (114) it will be seen that the last two can be expressed in the same form as (95), viz. (113) in the form

$$a_{H'(1)} = \lambda_{(1)} \frac{P_{CO_2}\alpha}{7{\cdot}60\beta} \quad \ldots\ldots\ldots\ldots\ldots\ldots(115)$$

and (114) in the form

$$a_{H'(2)} = \lambda_{(2)} \frac{P_{CO_2}\alpha}{7{\cdot}60\beta}. \quad \ldots\ldots\ldots\ldots\ldots\ldots(116)$$

Since

$$\lambda_{(1)} = K'_{1(1)} \frac{100 - q(1-d)}{100}, \quad \ldots\ldots\ldots\ldots\ldots\ldots(117)$$

$$\lambda_{(2)} = K'_{1(2)} \frac{100 - q(1-d)}{100d}, \quad \ldots\ldots\ldots\ldots\ldots\ldots(118)$$

$\lambda_{(1)}$ and $\lambda_{(2)}$ occur in (115) and (116). These are only constants as long as the factors in them do not vary or the variations neutralise one another.

$K'_{1(1)}$ and $K'_{1(2)}$ will only vary with the reaction if the activity coefficient of the bicarbonate ion varies with it.

L. Michaelis [1920] has lately drawn attention to this condition, regarding it however from the classical standpoint of Arrhenius, and therefore assuming that K'_1 varies with the degree of dissociation of the bicarbonate. His views appear in the form of a criticism on the theory which H. Straub and Klothilde Meier [1918–1920] put forward for the carbonic acid combining power of the blood on the strength of a series of experiments carried out by them. Straub and Meier's experiments and theories are however subject to an elementary error, and as will later be shown they are quite fallacious, but Michaelis' comments naturally do not lose their interest on this account.

According to Bjerrum's[1] theory there is reason to expect that the activity coefficient of an ion will decrease in a solution containing ions with an opposite charge and this will be especially the case if these ions are polyvalent or have a large molecular volume.

If there are already rather many ions present in the solution the effect of the new-comers will be small, and possibly in special cases it will not be noticed at all, but it is not right, without due consideration, to regard $K'_{1(1)}$ and $K'_{1(2)}$ as constants, as it is a fact that the ionic concentrations in phases (1) and (2) vary with the reaction.

The factor $\frac{100 - q(1-d)}{100}$ in (117) and the corresponding factor in (118)

[1] I have repeatedly had the opportunity of discussing this condition with Prof. Bjerrum about which he has up to the present only published a rough sketch.

will vary provided that q and d vary. From S. P. L. Sörensen's [1915–1917] experiments on egg albumin there is every reason to expect they will vary with the reaction, but what influence the variations will have on the "total constants" experimental investigations alone can inform us at present.

As will be shown in one of the subsequent chapters $\lambda_{(1)}$ in serum and the liquid phase of the blood corpuscles is constant within the limits of the reactions investigated (experimental errors are not very small in experiments with the fluids of blood cells), and we will therefore continue our calculations under this assumption.

Let $a_{H^\cdot}$ in the liquid phase of serum $= a_{H^\cdot(s)}$,

and $a_{H^\cdot}$ in the liquid phase of blood cells $= a_{H^\cdot(c)}$,

$\lambda_{(1)}$ in serum $= \lambda_{(s)}$,

$\lambda_{(1)}$ in blood cells $= \lambda_{(c)}$.

The volume of the blood cells expressed as a percentage of the total volume of the blood is Q. The partition of HCO'_3 between blood cells and serum is D, so that

$$\frac{C_{HCO'_3 \text{ in blood cells}}}{C_{HCO'_3 \text{ in serum}}} = D,$$

and lastly the mean concentration of combined carbonic acid in the blood expressed in volumes % = B, and

$$Q = \frac{\text{vol. of blood cells}}{\text{vol. of blood}} \times 100.$$

From (115) and (116) we then get

$$a_{H^\cdot(s)} = \lambda_{(s)} \frac{100 - Q(1-D)}{100} \times \frac{P_{CO_2}\alpha}{7{\cdot}60\,B}, \quad \text{.....(119)}$$

$$a_{H^\cdot(c)} = \lambda_{(c)} \frac{100 - Q(1-D)}{100D} \times \frac{P_{CO_2}\alpha}{7{\cdot}60\,B}. \quad \text{.....(120)}$$

(119) and (120) can be put in a form analogous with (95), viz.

$$a_{H^\cdot(s)} = \Lambda_{(s)} \frac{P_{CO_2}\alpha}{7{\cdot}60\,B}, \quad \text{.....(121)}$$

$$a_{H^\cdot(c)} = \Lambda_{(c)} \frac{P_{CO_2}\alpha}{7{\cdot}60\,B}, \quad \text{.....(122)}$$

$$\Lambda_{(s)} = \lambda_{(s)} \frac{100 - Q(1-D)}{100}, \quad \text{.....(123)}$$

$$\Lambda_{(c)} = \lambda_{(c)} \frac{100 - Q(1-D)}{100D}. \quad \text{.....(124)}$$

It follows from the above that in $\Lambda_{(s)}$ and $\Lambda_{(c)}$ four variables take part, of quite a different kind from those which determine K'_1 in (95), and it will thus be understood that we cannot immediately draw conclusions regarding the combination of carbonic acid in the blood from a numerical agreement between $\Lambda_{(s)}$ and K'_1 in any bicarbonate solution.

Fridericia [1920], Henderson [1908–1910], Hasselbalch [1916, 2], Parsons [1917–1920], Joffe and Poulton [1920], Michaelis [1920] and many others have not realised the full consequences of this condition (which in an almost

analogous way can be developed by the classical dissociation theory), and we are therefore compelled to investigate anew the questions mentioned in the introduction:

I. Can the hydrogen ion concentration of the blood be calculated by an equation similar to Henderson's and Hasselbalch's?

II. Is all the combined carbonic acid of the blood ionised?

I have attacked the first problem from a purely empirical standpoint. For practical work it is convenient to employ (121) in logarithmic form

$$p_{H^{\cdot}(s)} = p\Lambda_{(s)} + \log B - \log \frac{P_{CO_2 a}}{7{\cdot}60}, \quad \ldots\ldots\ldots\ldots\ldots\ldots(125)$$

and transform it thus

$$p_{H^{\cdot}(s)} = p\Lambda'_{(s)} + \log B - \log \int_0, \quad \ldots\ldots\ldots\ldots\ldots\ldots(126)$$

where $\int_0$ is the mean concentration of dissolved CO_2 in the blood expressed in volumes %, and

$$p\Lambda'_{(s)} = p\Lambda_{(s)} - \log \Phi_a\,(CO_2), \quad \ldots\ldots\ldots\ldots\ldots\ldots(127)$$

while $\Phi_a\,(CO_2)$ is the reciprocal of the relative absorption coefficient in blood.

The relative absorption coefficient in serum is 0·975 according to Bohr [1905] and 0·81 in blood corpuscles, from which $\Phi_a\,(CO_2)$ can be calculated by the following equation:

$$\Phi_a\,(CO_2) = \frac{100}{100 - \frac{19Q}{100} - \frac{2{\cdot}5\,(100 - Q)}{100}}. \quad \ldots\ldots\ldots\ldots\ldots\ldots(128)$$

RÉSUMÉ.

I. The modified Henderson-Hasselbalch equation is adapted for use with heterogeneous solutions.

II. It appears from the resulting equation that nothing can be concluded from the size of the total constant about the way the carbonic acid is combined, without taking the heterogeneity into account.

CHAPTER IV

METHODS FOR THE ELECTROMETRIC DETERMINATION OF THE APPARENT HYDROGEN ION ACTIVITY IN SOLUTIONS CONTAINING CO_2 WITH THE HELP OF THE PLATINUM HYDROGEN ELECTRODE, AND THE TECHNIQUE EMPLOYED.

The general principles and methods of estimation by the potentiometrical method have been often described and are to be found in every handbook of physical chemistry. The method used here is that described in S. P. L. Sörensen's *Études enzymatiques*, II [1909–1910] and those interested are referred to this work for further information. Within the limits of reaction obtaining in the following experiments the reaction of a solution will be a function of the CO_2 tension with which it is in equilibrium. What effect a change of the CO_2 tension

will have on the apparent hydrogen ion activity cannot be expressed in a general way, because the buffer action of the solution will determine it.

If it is desired to determine the reaction in a solution in equilibrium with a certain CO_2 tension, care must be taken that the CO_2 content does not vary during the measurement. There are two ways of avoiding this, either by saturating the solution with a hydrogen-CO_2 mixture (Höber's principle, 1903), or by bringing a very limited amount of hydrogen in equilibrium with the solution and taking care by suitable means that the CO_2 tension in the hydrogen only very slightly departs from that existing originally in the solution (Hasselbalch's principle, 1910), the potential of a platinum plate in contact with the solution being then measured.

For didactic reasons it is convenient to first describe Hasselbalch's principle. It is a development of methods with "stationary hydrogen" first used by Farkas in 1903 and later modified in different ways, see for example Botazzi in Neuberger's *Die Harn*.

Farkas allowed the liquid, the reaction of which he wished to estimate, to come into equilibrium with a quantity of confined hydrogen by diffusion through its surface, and he then measured the potential of a platinum plate dipping into the liquid. K. A. Hasselbalch showed however in 1910 that equilibrium is not reached in the time occupied by the experiment by this method, and as it was impossible to increase the duration because changes would occur in the blood he modified the method in such a way that the hydrogen was quickly brought into equilibrium with the liquid by rocking the electrode vessel containing the hydrogen and liquid a certain number of times. The liquid was then replaced by a fresh supply and the process repeated. Hasselbalch combined his method with the device of Michaelis and Rona [1909] which consisted in minimal immersion of the platinum in the liquid. Michaelis and Rona had demonstrated that by this refinement the depolarising action of the oxygen could be avoided or at any rate reduced to a minimum.

Konikoff [1913], who also worked at the "oxygen error," realised that the rocking must be continued until all the oxygen is reduced by the hydrogen (with the help of the platinised platinum). This is however hardly practicable in blood on account of the slow speed of the reduction (both electrode and blood become changed during the experiment). But there is no great difficulty in removing the free oxygen from inorganic salt solutions, (human) urine and similar liquids if a sufficiently large platinum plate is used, but a platinum wire of the size usually employed (Michaelis [1914], Hasselbalch [1916, 2] electrode at the bottom of Fig. 3) cannot remove the oxygen from solutions. Höber [1914], Peters [1914] and T. R. Parsons [1917] have also pointed out the possibility of depolarisation by O_2 in Hasselbalch's measurements and they recommended estimations on Höber's principle.

It is easy to convince oneself of the truth of the above by measuring the reaction of a phosphate mixture by Hasselbalch's method. In a vessel con-

taining a platinum plate electrode the liquid can be renewed several times without the potential practically varying at all, as Hasselbalch [1910 and 1911] showed a long time ago. But if the estimation is carried out in a vessel containing a platinum wire as electrode the potential[1] will fall on renewal (in the electrode vessel illustrated in Fig. 3 the potential fell about 2 millivolts for each renewal of liquid). This is due to the fact that besides H_2 and CO_2 the gas becomes contaminated with O_2 which depolarises the H_2–Pt electrode. No information is known to me in the literature about the activity of small quantities of O_2 in this connection. In measurements with an electrode vessel on Höber's principle, to be described later, I have obtained a rough idea of the magnitude of this factor. O_2 below 2 mm. Hg does not give an error of 1 millivolt, 3 mm. gives about $1\frac{1}{2}$ millivolt, and 8 mm. gives a large error if the potential otherwise remains constant. I can also confirm the frequent experience that no conclusion from the way in which the potential becomes established can be drawn about the presence of traces of O_2 in the electrode hydrogen.

Until 1916 Hasselbalch employed a small platinum plate as an electrode, while Michaelis used a platinum wire. In 1916 Hasselbalch adopted a hook-shaped wire for blood estimations, the point of which was allowed to touch the side wall of the vessel just over the surface of the liquid, and in this way he obtained minimal immersion.

According to experience at the Finsen Institute therefore wire electrodes should be used for liquids containing haemoglobin, and platinum plates for other liquids. In estimations of blood there are four points in particular to be noted:

(1) Contact between platinum and liquid should be minimal.

(2) The electrode must not be moved while waiting for the potential to become constant.

(3) The three-way tap must not become fixed.

(4) There must be no air bubbles in the connecting tube to the KCl solution.

We will now quite shortly consider a few points of importance in making measurements by Hasselbalch's method, neglecting the depolarising action of the oxygen, but remembering that if we did not do this the potential would be lower (and p_H smaller). We will assume also that the volume and pressure of the gas do not vary during the determination.

If a limited quantity of liquid, saturated with a gas at a given tension, which forms no combinations that split off the gas under the conditions of experiment, *e.g.* bicarbonate, is allowed to come into equilibrium with a limited space occupied by a gas, it can easily be shown[2] that at a given tem-

[1] By the potential is understood, here and henceforth when not otherwise stated, the total potential of the chain of concentration cells.

[2] See for example D. D. van Slyke and Cullen's [1917] equation for the calculation of the amounts of CO_2 in the apparatus of van Slyke.

perature the same proportion of the dissolved gas always goes over into the space, so that its partial pressure in the space will always be the same fraction of the pressure with which the liquid was originally in equilibrium. If the space is now allowed to come into equilibrium with a fresh amount of liquid, the partial pressure of the gas will rise further, but it will again be a definite fraction of the original pressure irrespective of its absolute magnitude.

It will be seen from equation (95) that $a_{H'}$ is proportional to the CO_2 pressure, from which it follows that the error, occasioned by the CO_2 tension in Hasselbalch's electrode being too low, will be independent of the absolute CO_2 tension and will only be dependent on the relation between the volume of liquid and gas and on the absorption coefficient of the investigated liquid for CO_2.

The statement of Hasselbalch [1910, 1911] that more renewals of the liquid should be made in the case of higher CO_2 tensions than with lower to obtain a constant potential, is due to the fact that a slight alteration of the H_2 tension has hardly any effect on the potential, while a large change greatly affects it (see equation (53)).

Neither Hasselbalch, Michaelis nor any other person who has worked with the method, corrected for the decreased H_2 tension (the correction is usually small), but Hasselbalch came to the conclusion that the best results are obtained by omitting this correction. It follows from what has gone before that we ought to be very cautious about admitting measurements by Hasselbalch's method as standard ones, but they can legitimately be used for comparison with one another if they are done with the same electrode and the CO_2 tensions do not differ very much from one another.

Similar considerations apply to blood, as the CO_2 split off does not greatly disturb the conditions but rather tends in favour of equilibrium being attained more easily than in bicarbonate solution. It appears that Hasselbalch and his collaborators found different values for $p_{H'}$ at different times. This is perhaps most evident from the fact that Hasselbalch and also Michaelis until 1916 held that blood with 40 mm. CO_2 had about 0·20 $p_{H'}$ greater at 18° than at 38°, but in 1916 when he changed the electrode he found the same $p_{H'}$ value at 18° as at 38°.

It is also worth while recounting a few experiences we have had at the Finsen Institute with regard to measurements of blood in the Hasselbalch electrode, which have also been noticed in numerous other laboratories. When the change of potential is followed after rocking it will be noted that it rises to a maximum and then slowly falls a little (the destruction of the electrode by the proteins). We have always taken the maximum reading as the correct potential.

When the electrode vessel is ready for an estimation the blood corpuscles sink, leaving the platinum in contact with serum alone. This is unquestionably of no small importance in the estimations, a view which is shared by many others. McClendon [1917] has even devised an electrode in which the blood

corpuscles and serum are separated by centrifuging before the estimation is made; and T. R. Parsons [1917] has saturated the blood with a mixture of H_2 and CO_2 and then centrifuged at the temperature of saturation (38°), keeping the saturation constant, and he showed that the potential of the separated serum was the same as that of the blood.

The other technical points are the same as those met with in measurements with Höber's electrode, to which the reader is referred.

Höber's principle, as already mentioned, depends on first bringing the liquid into equilibrium with a mixture of hydrogen and CO_2 of known constitution, and it is tacitly assumed that the oxygen is expelled by the saturation.

Numerous electrode cells have been constructed on this principle (*e.g.* by Bjerrum and Gjaldbaek [1919], Höber [1903], Peters [1914], McClendon [1917], Milroy [1917], Walpole [1913] and others). There can be no doubt that the measurements made on this principle should be corrected for diminished hydrogen tension, but there are no difficulties arising from the depolarising action of the oxygen (see however later measurements of the blood cell fluids).

General Technique.

The gases used were stored in the ordinary commercial metal cylinders. The hydrogen was prepared electrolytically and the cylinders contained as a rule about 99 % H_2 and about 0·5 % O_2. Once it was strongly contaminated with atmospheric air.

The hydrogen was led through palladium-asbestos kept red-hot in an electric oven. On account of the war I had only about 2 g. of 25 % palladium-asbestos at my disposal. It was packed in a combustion tube between asbestos corks. With this device I could get rid of the oxygen in 1 litre of hydrogen in a minute. The hydrogen was first washed with 5 % sulphuric acid and then with concentrated aqueous sublimate solution. Hydrogen prepared in this way contained about 99·5 % H_2.

The gases were mixed in a spirometer. For the hydrogen mixtures a 20-litre spirometer with water sealing was used, and care was taken that oxygen never gained access to it, a precaution it is absolutely necessary to observe. If the spirometer was not used for several days it had to be washed with O_2-free air several times. In addition to this spirometer two others of 125 litres were employed. In using a spirometer with water sealing it is important to recognise that gas mixtures containing CO_2 slowly change because CO_2 diffuses out through the water. The best way to avoid error is to take care that the CO_2 content of the gas mixture only differs slightly from the mixture that was previously in the spirometer, so that in investigating the effect of different tensions of CO_2 on a solution we start with the weakest or the strongest mixture and let the next nearest mixture follow on.

I have convinced myself a large number of times that the same gas mixture

is obtained from the spirometer (20 litres) if 9 litres are removed at the end of half-an-hour or 18 litres at the end of an hour. Only very occasionally have I found slight differences. In working with the large spirometers I have always taken the precaution to prepare 100 l. of the mixture and only withdraw 60 l. for the experiment. In this way the same mixture will be obtained after the lapse of one hour as after the lapse of four hours. Occasionally however I have noticed a slight fall in the CO_2 tension. Mixtures which greatly differed from the one which was last in the spirometer have usually been prepared on the evening before they were to be used. The mixing in the spirometer took place simply by moving the bell up and down. With the 20-litre spirometer, 25 times was sufficient; with the large one, 50 times was necessary. The gas mixture was conducted from the spirometer through a wash bottle which often contained 0·9 % NaCl when I was working with blood and strong salt solutions, otherwise pure water. I have however not strictly adhered to this but no appreciable error can have arisen from this cause. Before reaching the wash bottle in the thermostat the gas passed through a lead tube 1·5 metres long and about 0·3 cm. diameter so that it should attain the correct temperature. A Roux thermostat was employed (see Fig. 2). As the temperature in the upper part was often several degrees higher than in the lower, the perforated copper plates were replaced by wide-meshed galvanised iron netting and the netting covering the floor was raised somewhat. Under this a stirrer was installed which was driven by a small electric motor.

The stirrer[1] is illustrated in detail in Fig. 5 (p. 193). Its springs were similar to those of a dentist's drill (Claudius Ash and Co.). When it revolved at the proper speed and had the right inclination the temperature could be kept constant in the thermostat to within 0·5°. The conducting wires to the electrodes were led through the wall of the thermostat in paraffined glass tubes which were fixed in paraffined corks.

For the sake of economy in space it was necessary to place the thermo-regulator somewhat lower. In addition to the stirrer, the wires and the thermo-regulator an axle pierced the wall of the upper part to rotate the saturator and another the wall of the lower part to rock the Hasselbalch electrode. Both axles were worked with the help of a small electric motor.

The *saturators* used for small amounts of liquid were composed of 250 cc. bottles like those Hasselbalch [1916, 2] has used, but the inlet tube was not fitted with the piece of rubber tube which acts as a valve. In such an instrument 4–12 cc. of liquid were rotated about 60 times a minute for half-an-hour and about 1 litre of gas blown through every three minutes, at the same time. Under these conditions equilibrium was always established except in a very few cases with high CO_2 tension and very viscous liquids and when preliminary

[1] This and various other items of the apparatus used were constructed by the mechanic of the Finsen Institute, the late Mr Eriksen, after consultation with me and my best thanks are due to him.

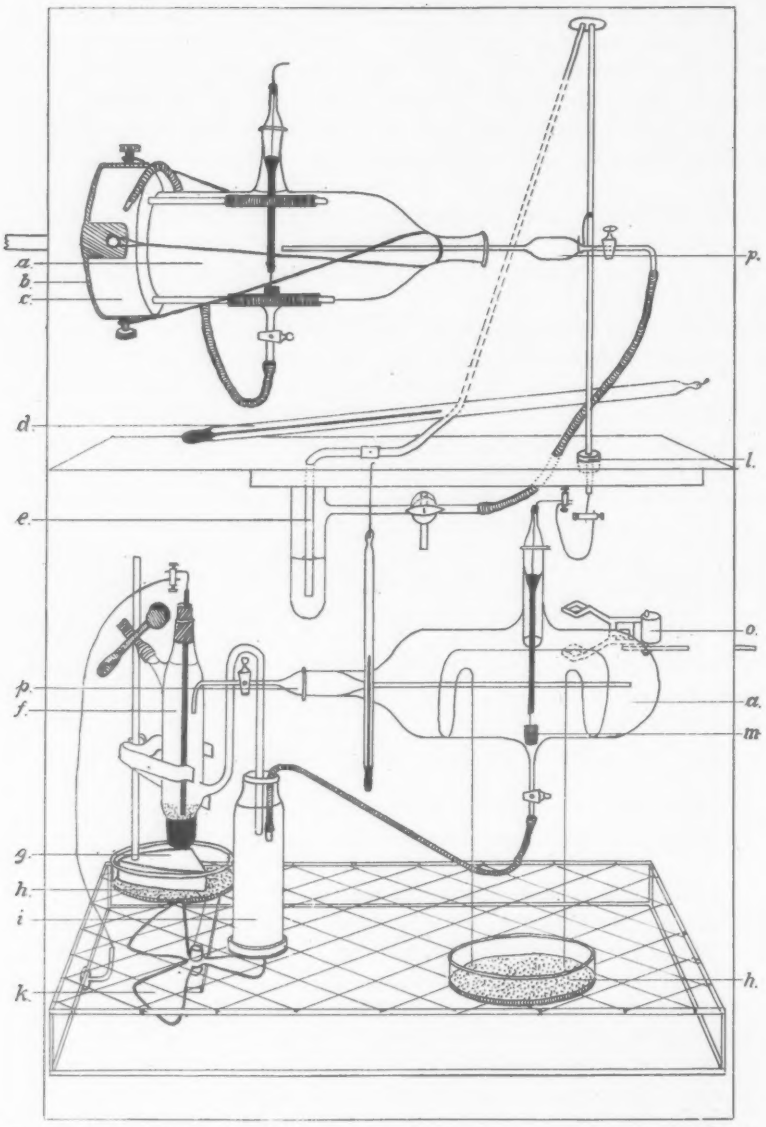


Fig. 2. The thermostat employed. In the upper department a small saturator electrode is rotating; in the lower, a similar one is ready for the estimation. *a*, small saturator electrode vessel; *b*, metal holder for the same during rotation; *c*, hollow wooden block; *d*, thermometer; *e*, wash bottle; *f*, calomel electrode; *g*, stand for calomel electrode; *h*, Petri dish filled with sealing-wax; *i*, vessel containing 3·5*m* KCl solution; *k*, fan for mixing the air in the thermostat; *l*, paraffined cork; *m*, platinum electrode; *o*, stand for Hasselbalch electrode; *p*, ground joint for tap.

treatment with pure CO_2 had been undertaken, but the difference was only slight. The experiment below will show the maximum error in the first case. Preliminary treatment with CO_2 in the small saturator was only employed in experiments where absolute accuracy was unimportant for the conclusions drawn, while it should be remembered that some of the CO_2 combinations may be a few vols. per cent. too low with tensions above 350 mm. CO_2.

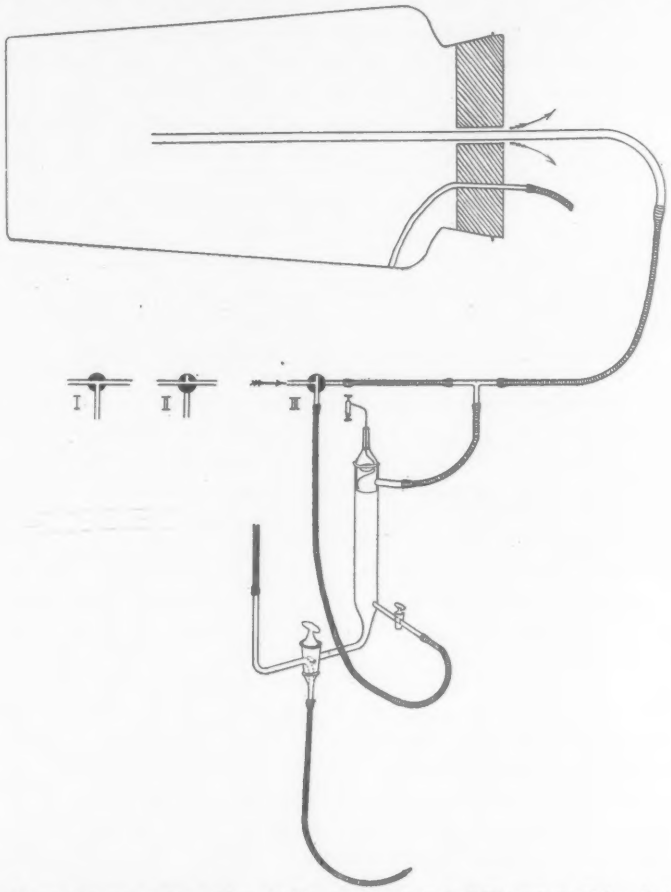


Fig. 3. Above, a large saturator; below, the Hasselbalch electrode E VI used as a Höber electrode. The tap is in position III during saturation and in position I when the liquid is transferred from the saturator to the electrode.

Ox blood containing 22·7 vols. % combined O_2 (concentrated by centrifuging) was treated with pure CO_2; half-an-hour after there were 178·8 vols. % total CO_2 in the blood; after a further treatment with CO_2 for $2\frac{1}{2}$ hours 183·4 vols. % CO_2 were present.

For larger amounts of liquid a saturator of the form illustrated in Fig. 3 was used. The capacity was a litre. A curved tube for extracting samples and a conducting tube for the gas were led through the cork in a similar manner to the small saturator. In a number of the experiments a stirrer was installed in the vessel, rotating with it. The frame of the stirrer was of heavily gilt brass and its blades of wood. It became evident however that the stirrer was superfluous. As equilibrium was always established in half-an-hour with 70 cc. in the saturator without the stirrer I dispensed with it afterwards, but rotated for an hour at the same time blowing about a litre of gas through every three minutes, total amount about 20 litres.

The saturator was rotated by being mounted in a holder of practically the same type as that illustrated in Fig. 2 (without wooden blocks), and fixed in it by means of straps.

The *electrodes* used were on Höber's principle and five kinds were employed.

I. Small saturator electrode[1]. This electrode which rather resembles a form described by McClendon [1917] was constructed as a development of the above saturators. The details of its structure will be understood from Fig. 2 in which an electrode is seen which is rotated in the upper part of the thermostat while gas is blown through it, and in the lower part an electrode ready for an estimation in a little wire basket fastened in a Petri dish with sealing-wax.

The electrode consists of a vessel with a rounded end. Its capacity is about 250 cc. In the middle a small funnel is blown which is continued into a thin glass tube with a small glass tap having a single hole. On the end of the tube a piece of rubber tube is placed which partly serves for taking samples of the liquid and partly for making a connection with the KCl solution. In the plane of the glass tube at right angles to the long axis of the vessel the outer part of a ground joint is blown in the side of the vessel and it is prolonged about a cm. through the wall. The axis of the ground joint and that of the glass tube form an angle of about 150° with one another and the axis of the ground joint cuts off a sector of the cross section (not looking towards the axis of the vessel). In the ground joint there is an inner part which is continued as a glass tube through the end of which a 0·5 mm. platinum wire passes. The wire carries a 0·5 × 1 cm. platinum plate of about 0·5 mm. thickness. Connection with the sealed platinum wire is made by means of mercury or by welding a copper wire on to it. The neck of the vessel also consists of a ground joint the internal half of which inside the vessel is prolonged into a tube and outside bears a single bored tap.

The electrode vessel in the upper portion is fixed in a hollow block of wood in which three 5 mm. strong rubber coated iron wires are fastened. The block of wood is held against an iron plate by means of straps which go round the neck of the vessel and fasten on to brass knobs attached to the iron plate.

[1] The electrode vessels were made at Jacob's technical glass works, Copenhagen.

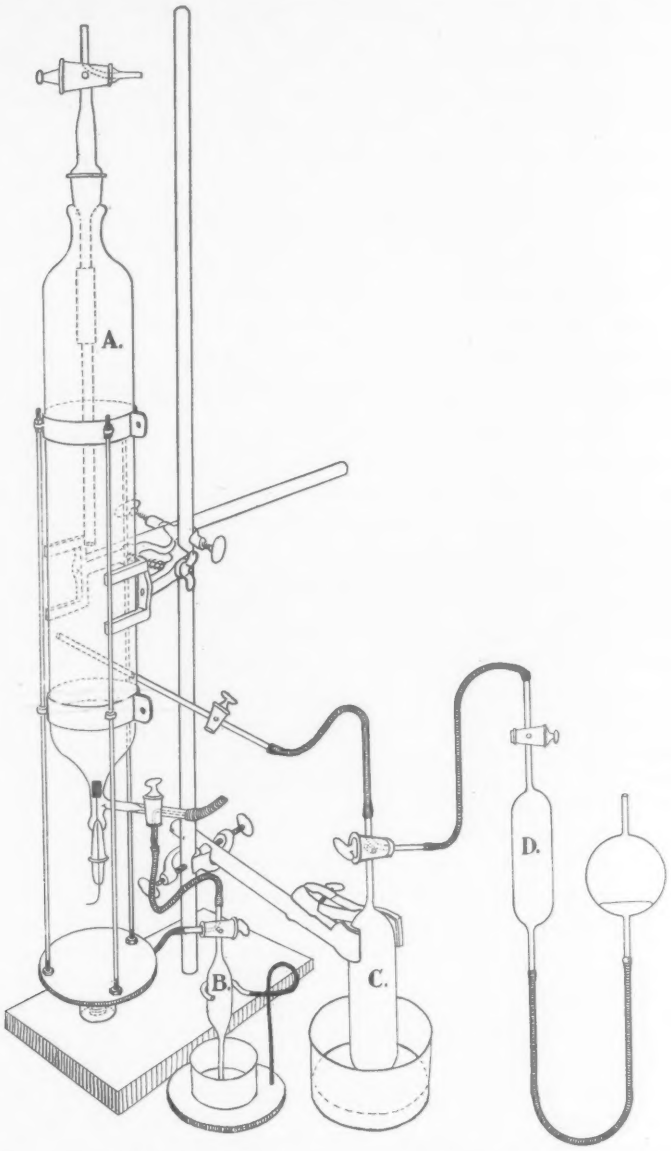


Fig. 4. On the extreme left is the large saturator L II in its stand after the potential has been measured. Then comes the small receiver filled with mercury for taking samples of the liquid. Following this is seen an "Ente" and in connection with its tap there is an ordinary gas receiver.

On the opposite side to the wooden block the iron plate carries a box which fits into a screw thread in the axis of the rotator.

The ground joints are lubricated with vaseline. The exit taps are not greased. In the electrode vessel 10 cc. of liquid are placed and rotated for half-an-hour while blowing through 8–10 litres of the H_2–CO_2 mixture. Samples of the liquid are taken while the introduction of the gas mixture continues, in a little mercury filled receiver which is seen in Fig. 4 (p. 189). The exit tap is then closed and afterwards the inlet tap, and the outer part of the ground joint is put in its place in the neck. The connecting tube is removed and the electrode placed ready for the estimation in the lower portion of the thermostat, the tube dipping into a vessel of 3·5*n* KCl and a connection made with the copper wire by a binding screw.

In constructing such an electrode again it would be an advantage to replace the small straight-bored exit tap by a tap with tail boring, as the conductivity through it is better.

In cases where the carbonic acid binding capacity of the liquid was known no samples were taken, and only 3–5 cc. were therefore introduced into the electrode vessel. In working with this saturator electrode and the saturator described above, samples of the gas blown through were taken immediately after the saturation was completed.

Now that the small saturator electrode has been described in detail it will only be necessary to give a short account of the other electrodes employed.

II. Large Saturator electrodes. (L I and L II.)

The electrode vessel was, on the whole, of the same type as the previously described smaller electrode but it was much longer and had a capacity of 1050 cc. The exit tap was an ungreased 3-way tap with tail boring (the straight boring being filled with paraffin). The inlet tap was a 3-way tap similar to the one fitted to the evacuation receiver (see Fig. 5, p. 193, upper row of taps) and where the neck of the vessel (the ground joint) joined the body a thin tube was sealed in at an angle of 45° with the axis of the vessel. This tube which was used for taking samples of the liquid had a small greased single-bore tap. The electrode vessel was mounted in a similar stand to that shown in Fig. 4, which carries the electrode vessel shown there. This stand had a box with a screw clamp and could be fixed to a rotating axle.

In measuring haemoglobin solutions (haemolysed blood) the following procedure was adopted. 20 cc. of the liquid was put in the electrode vessel and rotation started. Then either a strong stream of CO_2 was sent through it for a quarter to half-an-hour, or this preliminary treatment was dispensed with and 17 to 18 litres of H_2–CO_2 mixture passed through the electrode for half-an-hour. Then the ground joint was put in position and the inlet tap closed, and the electrode was rotated for half-an-hour with closed tap. A similar gas mixture was again passed through the electrode while rotating and finally the electrode was again rotated for half-an-hour with closed tap.

The stand was then removed from the rotating axle and hung up by silk

threads, so much liquid being sucked out that the exit tube was filled, and then the rubber tube to the tap was filled with KCl solution making a connection for the measurement in the usual way (the potential was not affected beyond the experimental error (1 millivolt) by further rotation). After the estimation was completed the electrode was put in such a position that its long axis formed an angle of about 45° with the horizon and the inlet tap was lowest. The latter was now put in connection with a receiver for taking samples of gas and its two inlet tubes filled from the receiver with mercury. Through the small glass tube a sample of the liquid was taken; and then a sample of gas for analysis, through the inlet tap. In this way there was a control on the equilibrium by comparing the analysis of the gas mixture used for the saturation with that in the electrode.

The same electrode vessel (L I) was used for an investigation of the liquid in equilibrium with low tensions of CO_2. The electrode was filled with pure H_2 and then the $H_2 + CO_2$ saturated liquid was introduced while H_2 was blown through the vessel, and the ground joint was then placed in position and it was rotated for one hour. As it seemed desirable not to allow contact between the haemoglobin solution and the platinum plate to take place before the measurement was actually begun, the electrode vessel shown in Fig. 4 (L II) was constructed. Its capacity was about 1050 cc. and it consisted of a glass tube with a ground joint in each end. The inner portion of the small ground joint carried the platinum electrodes; on the prolongation of the outer portion a side tube was joined with an ungreased 3-way tap with tail boring, with the help of which communication with the KCl solution and the removal of samples of the liquid could take place. A tube for taking samples of gas was sealed in the wall of the vessel and prolonged almost to the opposite side. This tube had a straight-bored tap. By means of the contrivance which is to be seen on the drawing the tube and the 3-way tap were rinsed with about 10 cc. gas from the electrode before the sample was admitted to the receiver.

It is useful to let the platinum wire in the electrode project 2 cm. on either side of the point where it is sealed into the glass. The other details will be appreciated with sufficient clearness from the diagrams.

The technique of saturation was the same as with the electrode L I.

III. Electrode vessels of the Hasselbalch type were likewise used but with H_2–CO_2 mixtures. One was the electrode shown by Hasselbalch and Gammeltoft [1915] and was called E III. It was only used for salt solutions. A little liquid was introduced and an H_2–CO_2 mixture bubbled through. Connection with the KCl solution was made by filling the 3-way tap and exit tube with KCl solution. The gas mixture was passed through until the potential became constant, the ground joint was turned and the 3-way tap adjusted in such a way that the bore of the tap was in line with that of the electrode vessel after which the potential was measured again.

The other electrode vessel of the Hasselbalch type called E VI was that

illustrated in Fig. 3. It will be seen that there is a $CaCl_2$ tube with a ground joint from which the handle is removed, a platinum wire through the top of the joint being substituted. The wire is coiled with a single turn in the interior of the ground joint and is bent in the form of a hook against its wall. The electrode vessel is washed out before the estimation with the H_2–CO_2 gas mixture and the gas is then led through the saturator as shown in Fig. 3. As soon as saturation is complete the stream of gas is turned off and the gas is conducted, by turning a T-tap to the position marked in the illustration, directly out into the saturator. The electrode vessel is then filled with liquid to the side opening in the ground joint avoiding the introduction of air into it and the measurement made in the usual way.

In using all these electrodes it must be remembered to wash out the tube and taps with the gas mixture in order to displace any air present.

As *connecting liquid* 3·5*n* KCl was used.

As *standard electrode* a *n*/10 KCl calomel electrode was prepared according to Ostwald and Luther's directions. For a large number of the estimations a calomel electrode and KCl solution was used which with long standing had become too concentrated giving an error of 2 millivolts, but this has been allowed for. Later four new electrodes were made which agreed very well with one another and numerous measurements of S. P. L. Sörensen's [1909–1910] phosphate solutions gave results which did not deviate 1 millivolt from Sörensen's own value. It is however probable that the stated value of p_H is 0·008 too low which corresponds to an error of $\frac{1}{2}$ millivolt. There is possibly thus a systematic error running through all the results but I have not corrected for it as it falls within the errors due to the method of regulating the temperature which I employed. A *Weston cell* was used as a *standard cell* and was tested twice during the investigations.

The *gas analysis* was carried out partly with the analysis apparatus of Haldane modified by A. Krogh and partly with a Petterson, Bohr, Tobiesen apparatus. CO_2 was absorbed in 50 % KOH, and O_2 in strongly alkaline pyrogallic acid solution. The maximum accuracy with Haldane's apparatus was 0·01 %; with Petterson's 0·02 %.

The gas content of the liquid was determined by exhausting with a Töpler-Hagen-Bohr mercury gas pump and analysing in Petterson's apparatus.

The liquids were collected in the little receiver shown in Fig. 4, the 3-way tap being rinsed with the liquid from which the sample was taken by suction, and the volume of the liquid being determined by weighing the mercury that had run out.

The evacuation receiver had a 3-way tap, like those shown in the uppermost row in Fig. 5. The sample was transferred to it by filling the dead space with distilled water and turning the taps as shown in the diagram (from left to right). It was necessary, as indicated, to have the tap closed with water and mercury during the pumping.

The extraction of gas from blood and amino-acids took place with a

saturated solution of boric acid in the receiver while in the case of inorganic salts 5 % sulphuric acid was used as a rule. The receiver was usually heated during the process in a water bath at 38°–45°.

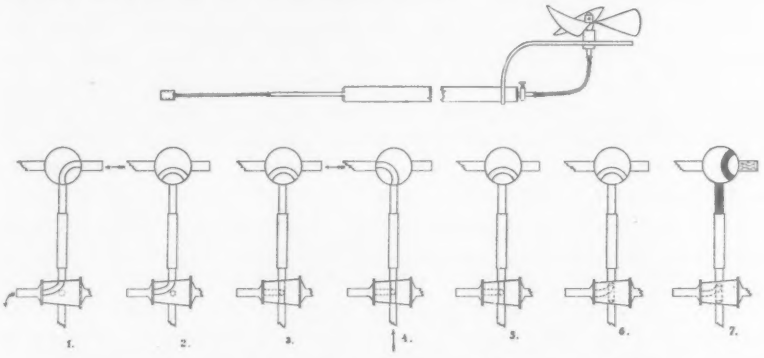


Fig. 5. Below is the small (liquid) receiver in connection with the inlet tap of the evacuation receiver. In the first position (from the left) the dead space is filled with distilled water, in the fourth position the liquid is led into the pump and in the last position the tap of the evacuation receiver is closed and the small receiver can be removed and pumping begun. Above is the stirrrer for the thermostat.

CHAPTER V

THE DIRECT EMPIRICAL DETERMINATION OF $A_{(s)}$ IN THE MODIFIED HENDERSON-HASSELBALCH EQUATION IN ITS APPLICATION TO BLOOD.

The values obtained in the present chapter are calculated from experiments in which the apparent hydrogen ion activity (H_2–Pt electrode) and the combined carbonic acid were estimated on the same sample of blood, and in which the CO_2 tension with which the blood was in equilibrium was known. In the literature similar estimations by K. A. Hasselbalch [1916, 2], T. R. Parsons [1917] and J. F. Donegan and T. R. Parsons [1919] will be found.

Hasselbalch's measurements were made with a small hook-shaped wire electrode. The volume of the hydrogen was about half that of the liquid in the electrode vessel used. Hasselbalch reckoned his constants from these experiments but did not correct for depression of hydrogen pressure. The constants have been recalculated by means of the equations given in chapter III, and the values obtained by correcting for decreased hydrogen pressure are given on the assumption that the CO_2 tension in the electrode vessel was the same as that in the saturation mixture and that CO_2 and H_2 alone were present in the electrode vessel. This proviso as we have seen in a previous chapter is

only approximately true as the liquid is usually not renewed in Hasselbalch's measurements in this electrode but it will be observed from the table that the correction is only small. Parsons' and Donegan and Parsons' measurements were done on Höber's principle with Walpole's electrode vessel and are already corrected for diminished H_2 tension.

There is reason to believe that the electrical measurements of Parsons and his collaborators are the best in the literature because they replatinised their electrodes each day and only let them come in contact with the blood when the measurement was about to commence, saturation being accomplished in a special saturator. Parsons made use of a water thermostat and possibly obtained a more constant temperature than we did at the Finsen Institute. But it seems likely that his estimations of combined CO_2 are a little uncertain. This assertion is supported by an examination of his experiments by plotting them as curves, and the view is also shared by E. Jarlöv [1919]. The electrical measurements recorded in Table VIII were carried out on serum which was centrifuged from blood saturated with CO_2, at the saturation temperature, saturation being maintained throughout. In experiments with reduced blood the saturation was carried out with H_2–CO_2 mixture and the serum was resaturated with the same mixture. In experiments with oxygenated blood saturation with CO_2–air mixture was performed and the serum separated by the centrifuge was resaturated with H_2–CO_2 mixture with the same content of CO_2 as in the mixture used for the blood. The measurements given in Table IX were made directly on H_2–CO_2 saturated blood. All Parsons' figures for combined CO_2 refer to blood.

I have myself performed a number of similar experiments most of which were carried out with the small saturation electrode, but some with low CO_2 tension with the large saturation electrode L I. The platinum electrodes were platinised before each measurement, which was found to be necessary (cf. Hasselbalch and Gammeltoft [1915]) otherwise the potential was lower than with freshly platinised ones. It was found unnecessary after a few experiments to heat the electrode to redness before platinising, but later on measuring strong haemoglobin solutions this was found to be essential, for which reason it would be advisable in future to avoid all trouble by always heating the electrode to redness first.

The potential always became constant a few minutes after the electrode was ready for an estimation (except at 38° when the air in the thermostat got cool on account of the unavoidable manipulations connected with the taking of samples etc.), and this was taken as one of the indications that everything was in order.

The relative absorption coefficient in Hasselbalch's, Parsons', Donegan and Parsons' and the author's blood experiments was always taken as 0·92, while in measurements of serum-rich and serum-poor mixtures of blood I have used 0·95 and 0·855 respectively.

When not otherwise stated the blood used in the present experiments

came from the cattle market and was not used before the day after the animal was slaughtered (so that "1st day" signifies the first day after slaughter). All the experiments with blood at room temperature are marked series A (and also a series with serum). They were all carried out in the autumn of 1918 and I have reason to believe all the potentials were too low without however being able definitely to state the cause. The mutual agreement within a series of experiments was however sufficiently good and therefore I have considered the experiments should be published; the course of the curve for the combination of carbonic acid is also of some interest.

The experiments marked B are subject to no such uncertainty.

Certain points about the value of $\Lambda_{(s)}$ can be surmised in advance. From the very considerable experience accumulated in different quarters on the reaction of the blood (serum) with various CO_2 tensions it can be concluded that the activity of the bicarbonate ion in serum cannot be greatly different from its mean concentration in the corresponding blood, from which it follows that $\Lambda_{(s)}$ must be of the same order of magnitude as K_1. Hasselbalch considered that K_I (Hasselbalch) varied with the mean concentration of bicarbonate in the same way as it varied in sodium bicarbonate solutions while Milroy [1917], Parsons [1919] and Michaelis [1920] on theoretical grounds thought the value ought not to vary in blood. I can support the opinion of these authors in so far as I have grounds for believing $\lambda_{(s)}$ does not vary but one cannot predict to what extent the variations of the volume of the blood corpuscles (Q in the equation (123)) react upon $\Lambda_{(s)}$ or whether the partition of the bicarbonate ion (D) between serum and blood corpuscles is variable. These conditions will be thoroughly investigated in a subsequent chapter. Haggard and Y. Henderson in 1919 calculated this constant from a number of Hasselbalch's and Parsons' experiments. The results differ somewhat from those about to be given chiefly because Haggard and Henderson have dealt with "hydrogen ion concentration" while I have dealt with "apparent activity" (Bjerrum), but furthermore these two authors have not realised that a number of Hasselbalch's experiments which they used in their calculations are really not valid for this purpose because the "hydrogen ion concentration" was not determined electrically, but reckoned from Henderson and Hasselbalch's equation.

In calculating the mean figures of the experiments I have felt justified in neglecting the values in brackets. This applies to the experiments of Parsons [1917] and Donegan and Parsons [1919] which refer to very low tensions of CO_2 because a small error in the determination of the combined CO_2 will produce a large error in $p\Lambda'_{(s)}$ in these cases. Further I have excluded all the exepriments of Donegan and Parsons where $p\Lambda'_{(s)}$ is under 6·10, since as a matter of experience too low values are much commoner than too high ones with the electrode cells employed, and the low values will therefore not be balanced in the mean value without the adjustment alluded to.

The correction for the temperature in my experiments at room temperature is effected in such a way that 0·005 was subtracted from $p\Lambda'_{(s)}$ at the

temperature of the experiment for each degree below 18° and added for each degree above 18°.

The mean of Hasselbalch's 24 determinations at 38° was

$$p\Lambda'_{(s)} = 6{\cdot}139 \pm 0{\cdot}0039^{1}.$$

From Parsons' and Donegan and Parsons' 48 determinations at 37°

$$p\Lambda'_{(s)} = 6{\cdot}178 \pm 0{\cdot}0039.$$

From my own 15 determinations at 38°

$$p\Lambda'_{(s)} = 6{\cdot}147 \pm 0{\cdot}0079.$$

Parsons [1917] and Donegan and Parsons [1919] have remarked that there is a difference between their $p_{H'}$ values and those calculated according to Hasselbalch's method. They write:

"It will be seen that the general direction of the curve ($p_{H'}$–P_{CO_2} curve) is the same but that Hasselbalch's blood appears to be more acid than mine at each CO_2 pressure. The reason for this may be to a certain extent in an individual variation, and without more data of this kind for the blood of a number of individuals the extent to which this factor operates must remain undecided. But the divergence may possibly be partly explained by differences in our experimental procedure. It is a significant fact that practically all errors which are likely to occur in electrometric determinations on blood (with the exception of loss of CO_2, which is out of the question in the experiments here described) tend to produce a reduction in the value of the E.M.F. with a consequent shift of the results to the acid side. The particular points in which Hasselbalch's procedure differs from ours is that he runs oxygenated blood into the electrode vessel, and so his results are liable to be affected by an error due to depolarisation of the electrode."

Parsons has repeatedly emphasised that blood is a heterogeneous system and he also realises that Hasselbalch's constant is a total constant, but in the article written in conjunction with Donegan they draw some conclusions which will not bear criticism. They write:

"There is no doubt of the applicability of Hasselbalch's calculations in homogeneous systems containing CO_2 and sodium bicarbonate in equilibrium. But while aqueous solutions of sodium bicarbonate and also blood plasma[2] represent such systems, the same is not true of whole blood. Here we have a two-phase system, composed of corpuscles and plasma in which the differences of composition of the two phases lead to marked differences of their combining powers for CO_2. Therefore the relation between CO_2-tension and CO_2-content in the whole blood will be different from that in plasma. Now it has been shown previously that it is the relation between free and combined CO_2 in the plasma, which determines the value obtained in an electrometric determination of $p_{H'}$ of the whole blood, while it is from the different relation

[1] The "mean error" of the mean determination.

[2] It follows from what was said in chapter III, that I am unable to support Donegan and Parsons on this point.

between free and combined CO_2 in the whole blood we shall obtain the calculated value of its p_H·. In other words the difference between the observed and the calculated p_H·'s of blood is simply an expression of the difference in behaviour of corpuscles and plasma towards CO_2."

Although I am in agreement with Parsons [1917] that the oxygen error may have caused Hasselbalch's measurements to be too low, he overlooks the fact that the CO_2 tension has not been properly appreciated in Hasselbalch's electrode (no renewal) which will militate against the O_2 error so that it is impossible to predict which factor will be paramount.

Donegan and Parsons also overlook the fact that Hasselbalch's constant was determined by measurements on whole blood so that the constant contains a correction for the different bicarbonate contents of serum and blood corpuscles, and that the calculated and measured p_H· must be the same if Hasselbalch's and Parsons' techniques give concordant results.

That the $p\Lambda'_{(s)}$ value of Parsons and Donegan and Parsons is 0·023[1] greater than mine can only be due to lack of uniformity in the technique of the electrical measurements and as they are all carried out on the Höber principle it seems to me that provided the difference is a real one there can only be two possibilities:

(1) either our calomel electrodes are different,

(2) or my lower value depends upon my not having glowed my platinum electrode before each platinising.

The difference between our constants is really very small, although it seems to be real judging from the mean error, and it would mean a considerable number of new experiments to determine what the true value ought to be.

If the tables are examined more carefully it will appear that $p\Lambda'_{(s)}$ from Donegan and Parsons' measurements seems to increase greatly with reactions more alkaline than p_H· 8·0. This is however doubtful and my own measurements with a marked alkaline reaction though few in number do not show this.

On the face of it there appears to be no certain variation of $p\Lambda'_{(s)}$ caused by change of reaction but a more careful investigation of the question might be useful.

If we divide Parsons' and Donegan and Parsons' measurements in such a way that all the experiments in which p_H· was equal to or over 7·46 are placed in one group, and all those in which p_H· was equal to or below 7·45 are placed in a second group, we get 21 experiments

$$p_H \geqq 7{\cdot}46 \quad p\Lambda'_{(s)} = 6{\cdot}188 \pm 0{\cdot}0063$$

with a standard deviation of 0·029; and 27 experiments

$$7{\cdot}45 \geqq p_H \quad p\Lambda'_{(s)} = 6{\cdot}170 \pm 0{\cdot}0049$$

with a standard deviation of 0·023. The difference between the constants is $0{\cdot}018 \pm 0{\cdot}008$.

[1] Corrected for the influence of the temperature.

It seems from the above as if $p\Lambda'_{(s)}$ increases a little with the p_{H^*} value. The standard deviation in the two series is of the same order so that we are justified in assuming there is a real deviation of the "constant," and the results group themselves about the mean in the same way in the two groups so that we can compare the mean values.

Dealing with my own experiments at 38° in a similar way, we have 8 experiments

$$p_{H^*} \leqslant 7{\cdot}39 \quad p\Lambda'_{(s)} = 6{\cdot}161 \pm 0{\cdot}0099$$

with a standard deviation of 0·028; and 9 experiments

$$7{\cdot}38 \leqslant p_{H^*} \quad p\Lambda'_{(s)} = 6{\cdot}133 \pm 0{\cdot}012$$

with a standard deviation of 0·036. The difference between the constants is $0{\cdot}028 \pm 0{\cdot}0155$; $p\Lambda'_{(s)}$ seems therefore to increase a little with the p_{H^*}. It is clear however from the calculations that the change in $p\Lambda'_{(s)}$ is small in all cases and a large number of experiments would still be necessary to accurately determine its magnitude.

At 38° the following is the mean of Parsons' [1917], Donegan and Parsons' [1919] and my own measurements:

$$p\Lambda'_{(s)} = 6{\cdot}159, \text{ from which } p\Lambda_{(s)} = 6{\cdot}193$$

and $\Lambda_{(s)} = 6{\cdot}41 \times 10^{-7}$.

At 18° I found $p\Lambda'_{(s)} = 6{\cdot}24$, from which $p\Lambda_{(s)} = 6{\cdot}27$

and $\Lambda_{(s)} = 5{\cdot}4 \times 10^{-7}$.

Résumé.

I. The constant $\Lambda_{(s)}$ has been calculated at body temperature and room temperature from all the suitable experiments to be found in the literature.

II. A considerable number of new experiments for the determination of the constant have been performed.

III. A small discrepancy has been shown to exist between the constant reckoned from Parsons' and Donegan and Parsons' experiments and my own. and the possible causes of the disagreement are discussed.

IV. It has been shown to be probable that the constant increases a little with increasing hydrogen ion activity.

Table VII. Calculated after K. A. Hasselbalch, *Biochem. Zeitsch.* **78**, p. 123.

	mm. Hg CO_2	Vols. % CO_2 (combined)	p_H·(s)	$pA'_{(s)}$	Corrected p_H·	Corrected $pA'_{(s)}$
38°. Ox blood I	39·3	42·6	7·31	6·11	7·32	6·12
	28·1	37·2	7·43	6·14	7·44	6·15
	63·1	47·9	7·19	6·14	7·21	6·15
	95·6	62·7	7·09	6·10	7·12	6·13
38°. Ox blood II	10·7	31·6	7·77	6·13	7·77	6·13
	33·6	47·8	7·47	6·15	7·48	6·16
	96·7	65·0	7·11	6·11	7·14	6·14
	61·2	58·7	7·27	6·10	7·29	6·12
	20·1	39·8	7·62	6·15	7·63	6·16
	43·7	51·8	7·36	6·11	7·37	6·12
	74·0	64·7	7·22	6·11	7·24	6·13
38°. Ox blood III	41·1	48·8	7·37	6·12	7·38	6·13
	41·0	48·5	7·37	6·13	7·38	6·14
38°. Ox blood V	42·7	44·3	7·31	6·13	7·32	6·14
	39·3	44·5	7·33	6·10	7·34	6·11
	40·9	42·2	7·29	6·12	7·30	6·13
38°. Ox blood VI	36·3	45·2	7·39	6·12	7·40	6·13
	54·4	52·0	7·32	6·17	7·34	6·19
						Mean 6·14
38°. Human blood (K.A.H.)	50·8	57·1	7·31	6·09	7·33	6·11
	45·7	52·9	7·37	6·13	7·39	6·15
	32·7	45·1	7·43	6·12	7·44	6·14
	18·5	35·1	7·60	6·15	7·61	6·16
	22·4	36·7	7·54	6·15	7·55	6·16
	80·7	61·3	7·17	6·12	7·20	6·15
						Mean 6·145
18°. Human blood (K.A.H.)	44·8	54·3	7·23	6·20	7·24	6·21
	22·3	44·3	7·47	6·22	7·48	6·23
						Mean 6·22

Table VIII. T. R. Parsons, *J. Physiol.* **51**, p. 448.

No.	mm. Hg CO_2	Vols. % CO_2 (combined) Oxygenated blood	Vols. % CO_2 (combined) Reduced blood	p_H· Oxygenated blood	p_H· Reduced blood	$pA'_{(s)}$ 37° Oxygenated blood	$pA'_{(s)}$ 37° Reduced blood
1	37·4	43·4	47·3	7·37	7·45	6·13	6·18
2	0·79	—	12·4	—	8·55	—	(6·19)
3	19·6	34·4	39·9	7·62	7·69	6·20	6·22
4	72·1	59·9	64·4	7·25	7·28	6·17	6·17
5	10·1	30·0	31·5	7·79	7·86	6·16	6·21
6	8·1	—	27·4	—	7·91	—	6·22
7	55·3	—	55·1	—	7·35	—	6·19
8	33·4	41·5	47·6	7·48	7·53	6·13	6·21
9	5·7	21·3	23·8	7·96	8·01	6·22	6·22
						Mean 6·17	6·20
						6·19	

Table IX. Calculated after Donegan and Parsons, *J. Physiol.* **52**, pp. 317–318.

mm. Hg CO_2	Vols. % CO_2 (combined)	p_H	$p\Lambda'_{(s)}$ 37°
0·7	4·5	8·29	(6·32)
9·7	33·1	7·78	(6·08)
29·6	44·3	7·51	6·17
44·0	53·1	7·39	6·14
65·7	60·7	7·30	6·17
1·3	6·4	8·26	(6·30)
12·7	30·5	7·75	6·20
37·0	45·0	7·45	6·20
67·6	52·6	7·24	6·18
2·1	8·4	8·26	(6·29)
18·8	36·3	7·67	6·22
31·5	48·2	7·49	6·14
2·2	10·4	8·30	(6·36)
18·1	39·4	7·68	6·16
38·9	43·8	7·44	6·22
59·3	53·9	7·30	6·18
1·3	7·7	8·19	(6·25)
20·0	35·5	7·59	6·18
42·7	45·1	7·36	6·17
65·9	53·0	7·24	6·17
1·3	10·0	8·27	(6·22)
15·9	35·6	7·66	6·15
38·2	46·9	7·41	6·16
51·5	50·7	7·31	6·14
1·0	12·4	8·28	(6·01)
21·3	38·5	7·58	6·16
40·5	46·8	7·37	6·14
60·0	50·8	7·26	6·17
2·0	8·9	8·15	(6·34)
14·2	29·3	7·67	6·19
30·8	38·9	7·43	6·17
45·9	45·0	7·34	6·18
2·0	8·5	8·23	(6·32)
19·1	33·5	7·61	6·20
40·8	44·6	7·39	6·19
60·7	54·9	7·27	6·15
10·9	26·0	7·74	6·20
29·3	37·8	7·47	6·20
45·3	46·2	7·32	6·20
0·7	8·8	8·02	(6·75)
11·6	27·4	7·58	(6·03)
31·3	37·0	7·37	6·13
46·1	41·3	7·27	6·15
12·5	23·0	7·61	6·18
25·6	30·3	7·42	6·18
40·9	36·9	7·28	6·16
			Mean 6·18

Table X. Determination of $p\Lambda'_{(s)}$ in blood at 38° with the small saturator electrode, Series B.

mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\Lambda'_{(s)}$	
13·6	0·4	27·7	0·2	7·65	6·18	
74·6	0·5	26·5	0·6	—	—	Human blood (E.J.W. 31. i. 19). Placed on ice immediately
39·5	0·4	42·8	0·6	7·39	6·20	
109·2	0·5	64·7	0·2	7·10	6·15	
544·5	0·5	99·5	0·1	6·60	6·17	In the ice safe overnight
	Ordinary air		24·8		Mean 6·18	
501·9	0·4	84·2	0·0	6·53	6·13	
83·5	0·2	55·7	0·1	7·07	6·08	
36·0	0·5	44·1	0·3	7·34	6·10	Ox blood
8·4	0·3	24·2	—	7·75	6·11	
108·7	0·2	60·5	0·4	7·07	6·15	
39·6 + ord. air		39·6	20·1		Mean 6·10	
33·4	0·3	44·3	0·2	7·43	6 14	Rabbit blood from four animals (quite fresh, placed on ice immediately)
120·9	0·4	65·4	0·2	7·04	6·14	
39·4	0·3	45·5	0·7	7·38	6·15	
37·8	140·0	41·2	13·2		Mean 6·14	
518·1	0·7	99·4	0·0	6·55	6·10	
39·6	0·3	49·8	0·7	7·45	6·18	Horse blood
97·3	0·5	65·7	0·5	7·16	6·19	
31·9	0·5	47·0	0·6	7·51	6·17	In the ice safe overnight
34·1	137·5	40·0	20·0		Mean 6·16	
26·0	1·0	36·3	0·1	7·48	6·16	Human blood, fresh (chronic nephritis)
19·9	0·2	—	0·1	7·59	—	
96·6	0·4	—	—	7·10	—	
36·4	136·8	34·7	20·8	—	—	

Mean of all the determinations 6·15.

Table XI. Determination of $p\Lambda'_{(s)}$ in blood at 18° with the small and large (L I) saturator electrodes, Series A.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\Lambda'_{(s)}$	
17·5	250·4	0·8	112·1	0·1	6·85	6·23	Human blood (E.J.W. 3. x. 18),
18·5	91·8	0·6	89·2	0·4	7·17	6·23	fresh, placed on ice
18	24·1	0·8	59·4	—	7·62	6·28	
18	26·3	0·8	56·5	—	7·60	6·32	2nd day
18	37·7	0·3	—	—	7·50	—	
18	16·6	0·8	48·9	0·2	—	—	
18	73·1	0·7	78·6	0·5	7·23	6·25	
18	58·0	143·8	65·6	22·7	—	—	
18	58·0	143·8	65·9	23·1	—	—	
18	4·2	0·8	26·3	0·8	8·02	6·29	3rd day. Large saturator elect. (L I)
					Mean	6·27	
16·5	147·1	0·8	74·0	0·2	6·84	6·21	Ox blood
17	80·2	0·6	65·3	0·4	6·07	6·22	
17	58·8	0·8	59·8	0·5	6·19	6·24	
17	58·8	0·8	59·6	0·0	6·19	6·24	CO_2 preliminary treatment
16·5	419·6	1·1	85·8	0·4	6·43	6·19	Next day. CO_2 preliminary
17	51·7	1·2	58·4	0·0	7·26	6·27	treatment
17	16·2	0·6	42·3	0·0	7·57?	(6·22)?	„ „
17	22·3	1·0	48·4	0·2	7·50	6·23	„ „
16·5	5·3	0·6	29·1	0·0	7·92	6·24	3rd day. Large saturator elect. (L I)
					Mean	6·24	
	Air from water blower			14·9			
	„		„	15·1			
19	77·1	0·8	68·5	—	7·15	6·25	Dog blood (0·1 % oxalate)
19	29·3	0·3	53·5	—	7·47	6·26	
19	56·4	0·3	62·4	0·4	7·29	6·29	
19	127·5	0·2	76·0	0·2	6·97	6·24	
19	337·6	0·5	88·8	0·2	6·65	6·26	
19	2·2	0·7	21·8	0·4	8·15	(6·38)	Large saturator elect. (L I)
					Mean	6·26	
	Air from water blower			19·2			
	„		„	19·2			
18·5	41·5	0·3	63·0	0·4	7·38	6·25	60′ after blood Rabbit blood
18·5	24·2	0·3	53·1	0·0	—	—	90′ was taken. (on ice)
18·5	83·1	0·5	76·5	0·2	7·14	6·23	150′ „
18·5	368·0	0·2	107·0	0·4	6·61	6·20	195′ „
18·5	44·2	0·5	64·1	0·4	7·35	6·24	240′ „ Vol. % O_2
18·5	16·0	0·3	48·6	0·5	7·64	6·21	300′ „ (combined)
18·5	81·7	0·4	73·8	0·1	7·16	6·25	390′ „ 16·6
					Mean	6·23	
19	91·5	0·6	83·5	0·7	—	—	1st day. Horse blood
19	22·0	0·3	56·2	0·7	7·62	6·26	
19	48·7	0·3	69·9	0·7	7·39	6·28	
18·5	3·0	0·4	25·2	0·4	8·14	6·26	L I
19	39·6	0·2	66·3	0·5	7·43	6·25	
18·5	484·7	0·2	112·2	0·9	6·54	6·25	2nd day
19	80·2	0·2	78·2	—	7·20	6·24	3rd day
					Mean	6·26	
	Air from water blower			22·3			
	„		„	22·9			
18·5	128·6	0·6	82·0	0·6	6·99	6·23	2nd day. Equal parts of horse
19	58·5	0·6	71·9	0·5	—	—	blood and serum. $\Psi = 5$
19	38·8	0·3	66·6	0·4	7·42	6·24	
21	269·8	0·4	90·3	—	6·72	6·24	3rd day
					Mean	6·24	
20	126·9	0·4	90·3	—	—	—	3rd day. Horse blood cell sus-
20	42·6	0·4	59·6	—	7·42	6·27	pension
20	72·1	0·2	74·3	—	7·26	6·25	
					Mean	6·26	

Table XI (*continued*)

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'}$	$p\Lambda'_{(s)}$		
19·5	536·4	0·4	102·2	—	6·45	6·22	2nd day	Ox blood
21	91·9	0·3	75·5	—	7·13	6·25	3rd day	
19·5	24·3	0·2	53·3	—	7·52	6·23	4th day	
—	—	156·0	—	18·96	Mean	6·23		
—	—	156·0	—	18·97				
19	510·5	0·5	89·6	—	6·44	6·25	2nd day	Equal parts of ox
21	91·9	0·3	72·5	—	7·08	6·23	3rd day	blood and serum.
20	34·3	0·4	60·7	—	7·45	6·25		$\Psi = 5$
20	24·3	0·2	57·8	—	7·53	6·23	4th day	
—	—	156·6	—	8·5	Mean	6·24		
19	508·0	—	115·8	—	6·59	6·24	1st day	Ox blood cell sus-
20	65·7	0·3	70·3	—	7·24	6·23	3rd day	pension. $\Psi = 14{\cdot}5$
20	34·3	0·4	57·1	—	7·42	6·26		
20	2·8	1·1	34·4	—	7·80	6·25	5th day	L I
—	—	156·6	—	33·8	Mean	6·25		
18	65·3	0·5	73·0	0·1	—	—	1st day	Ox blood
19	51·0	1·2	66·6	0·2	7·29	6·21	2nd day	
19	6·0	—	36·5	0·3	7·96	6·22	3rd day	L I
18	58·0	140·7	—	16·5	Mean	6·215		
18	58·0	140·7	—	16·6				
19	38·6	0·8	64·0	0·5	7·42	6·28	1st day	Blood and serum,
19	51·0	1·2	66·7	0·4	7·29	6·23	2nd day	equal parts. $\Psi = 5$
18	144·5	0·6	81·6	—	6·91	6·22	3rd day	
—	—	146·3	—	6·9	Mean	6·24		
18	53·7	1·0	71·0	0·9	7·33	6·23	2nd day	Ox blood cell sus-
18	64·9	1·3	76·7	0·1	7·24	6·19	3rd day	pension
18	64·9	1·3	75·4	0·6	7·24	6·20		
—	—	146·3	—	32·3	Mean	6·20		

CHAPTER VI

THE DETERMINATION OF THE $p\Lambda'_{(s)}$ VARIATION.

From equation (127) and (123) in logarithmic form we get

$$p\Lambda'_{(s)} = p\lambda_{(s)} - \log \frac{100 - Q(1-D)}{100} - \log \Phi_a\,(CO_2). \quad \ldots\ldots\ldots(129)$$

If we now calculate $p_{H'(s)}$ for blood by using the following equation

$$p_{H'(s)}\,(\text{uncorrected}) = p\lambda_{(s)} + \log B - \log \int_0 = p\lambda_{(s)} + \log \Phi_a\,(CO_2) + \log B - \log \frac{P_{CO_2 a}}{7{\cdot}60} \ldots\ldots(130)$$

instead of (126) $\qquad p_{H'(s)} = p\Lambda'_{(s)} + \log B - \log \int_0,$

where B is as usual the volume % of combined CO_2 in the blood and $\int_0$ is the dissolved CO_2 in blood, the p_H value is not correct because

$$+\left(-\log \Phi_a\,(CO_2) - \log \frac{100 - Q(1-D)}{100}\right)$$

must be added to it.

As will be seen from (129) this gives in addition the $p\Lambda'_{(s)}$ variation provided $p\lambda_{(s)}$ is constant, which *a priori* is very probable as the volume of the protein phase in serum is relatively small and the amount of salt large in comparison with the quantity of electrolytes which vary with the reaction.

$\Phi_a\,(CO_2)$ has been determined from (128) on the assumption that 100 cc. blood corpuscles combine with 49 cc. O_2. This is undoubtedly incorrect but the error involved in the above approximation is but trifling compared with the uncertainty of the relative absorption coefficient in blood corpuscles (0·81) [Bohr, 1905].

When these investigations were begun there were only a few rather uncertain experiments which allowed of the determination of D.

$$\left(D = \frac{\text{c.c. combined } CO_2 \text{ in 100 c.c. blood corpuscles}}{\text{c.c. combined } CO_2 \text{ in 100 c.c. serum}}\right).$$

Since then some experiments by L. S. Fridericia [1920] and by J. Joffe and E. P. Poulton [1920] have appeared from which this factor may be determined. The authors have themselves made the calculation which in many ways is similar to the one I am about to put forward, and they have also drawn attention to the difficulties with the Henderson-Hasselbalch equation which arise in connection with it. Joffe and Poulton have moreover shown how the difficulties can be evaded.

I have however made a recalculation of these authors' experiments because I believe the method of calculation I have employed has considerable advantages over that of Joffe and Poulton and it also better admits of a comparison of the various series of experiments than would otherwise be possible. A. Schmidt [1867] and N. Zuntz [1867] showed almost simultaneously in 1867 that the blood corpuscles contain considerable amounts of combined CO_2. They further proved that this amount rose rapidly with the CO_2 tension (although Schmidt on the basis of some poorer experiments from a technical standpoint, with dog's blood, considered that the CO_2 combination passed through a minimum with increasing CO_2 tension), and lastly they showed that the combination of CO_2 with serum which was obtained from blood saturated at high CO_2 tensions was considerably greater than with serum of the same CO_2 tension provided the serum was got from (the same) blood at lower tensions. They explained this phenomenon—which I shall repeatedly revert to and which I have called the Schmidt-Zuntz phenomenon—by assuming a transference of sodium from the blood corpuscles to the serum (plasma) under the conditions cited. The experiments do not permit D to be calculated with reasonable certainty. L. Fredericq [1878] has shown that in horse blood corpuscles a little over half as much CO_2 is combined as in the

serum at physiological CO_2 tensions, while at 745 mm. about nine-tenths are combined in the blood corpuscles as compared with an equal volume of serum.

From experiments carried out by Fr. Kraus [1898]—experiments which seem to me to be much too little known—it is clear that D in ox blood at physiological CO_2 tensions is about $\frac{3}{4}$, while at a couple of hundred mm. CO_2 it is about 1.

Quite recently W. Falta and Richter-Quittner [1920] have asserted that the partition of Cl, glucose and non-protein nitrogen between blood corpuscles and serum is absolutely different from that between blood corpuscles and plasma because they thought they were able to show that the blood corpuscles, before coagulation sets in, do not contain appreciable amounts of these substances, while after coagulation has taken place they contain considerable quantities—as is well known. They further claim to have proved that different treatment of the blood such as the prolonged action of cold, addition of oxalate, etc. has the same effect as defibrination. If Falta and Richter-Quittner's experiments are really sound they will be of epoch-making importance in the subject of the osmotic conditions of the blood corpuscles and the permeability of their surfaces, and pursued to their ultimate conclusion they will occasion a complete change of the current view concerning the conditions of equilibrium between blood corpuscles and plasma, and thereby influence our ideas on the production of lymph and kindred subjects.

L. Fredericq's [1878] experiments show that the partition of combined CO_2 between blood cells and serum is the same as between blood cells and plasma. Fredericq hindered the coagulation of the blood (horse blood) by preserving it in an excised jugular vein.

As early as 1893 H. J. Hamburger showed that the partition of chloride between blood corpuscles and plasma (obtained under oil) was the same as that between blood corpuscles and serum when the blood was defibrinated without admitting the air.

K. L. Gad Andresen [1920] has raised objection to the experiments of Falta and Richter-Quittner, as he has demonstrated that the partition of urea is independent of coagulation (hirudin-plasma). Rich. Ege [1920] has shown the same for chlorine and glucose. A. Norgaard and H. C. Gram [1921] have shown that the Cl content of the blood is a simple function of that of the blood corpuscles (or amount of haemoglobin) when it is assumed the corpuscles contain about half the amount of Cl present in the serum (per unit volume). They also found this ratio in blood rendered incoagulable by the addition of isotonic sodium citrate solution.

H. C. Hagedorn [1920] has shown in numerous experiments that human blood corpuscles obtained from blood with the addition of hirudin contain considerable amounts of glucose. In a short paper I have myself [Warburg, 1920] shown that chlorine and combined CO_2 are distributed in the same manner between blood corpuscles and hirudin plasma as between blood corpuscles and serum even though the CO_2 tension is varied greatly.

Falta and Richter-Quittner [1921] have maintained their position in opposition to these experiments of Gad Andresen, Ege, Hagedorn and Warburg, or at any rate have only modified it to a very slight extent as regards the main problem. It is however impossible to bring the two views into agreement with one another. There are two possibilities—either Falta and Richter-Quittner are unable to analyse the contents of the blood corpuscles with regard to the substances in question, or Fredericq, Hamburger, Gad Andresen, Ege, Norgaard and Gram, Hagedorn[1] and the author are unable to do it.

It may further be of interest to discuss an erroneous assumption which seems to be widespread.

N. Zuntz [1867, 1868] showed in the paper previously referred to, that the alkali in blood which was estimated by titration decreased on coagulation and concluded from this that acid was formed in the process. This fact has subsequently been confirmed several times, amongst others by Loewy and Zuntz [1894] and by A. Jaquet [1892], but it has been curious that the amount of acid formed varied comparatively greatly from one experiment to another. Quite recently Howard Haggard and Yandell Henderson [1920, 1] have again demonstrated the presence of such an acid, having found, at high CO_2 tensions, several volumes % less CO_2 combined in defibrinated blood than in oxalated blood (a difference which is however small compared with what Zuntz [1868] originally found). J. Joffe and E. P. Poulton [1920] have carried out numerous CO_2 determinations of Joffe's blood, defibrinated and oxalated, and they found no difference in the combined CO_2. A. Krogh and G. Liljestrand [1921] have also found the same amount of CO_2 combined in the defibrinated and oxalated blood of Liljestrand. Dr Chr. Lundsgaard and Dr E. Möller have kindly reported to me that in a number of experiments not yet published they have found no difference in the CO_2 combined in defibrinated and oxalated blood (man).

In the previously mentioned experiments of the author no difference in the combined CO_2 in defibrinated and "hirudin" blood (horse) was found. That some investigators discovered a decreased titrimetric alkalinity or CO_2 combining power after coagulation can undoubtedly be ascribed to the fact that a quantity of blood corpuscles may be removed by the defibrination, the bearing of which on the present problem has not hitherto been appreciated, as these at higher CO_2 tensions or more acid reactions function as a base (see chap. XI) and combine with acid.

L. S. Fridericia [1920] has reported three experiments with 0·1 % oxalated ox blood which permit of the calculation of D at three different reactions. The reactions were calculated from the plasma figures, the value for $p\lambda_{(s)}$ which will be found below being used.

Hasselbalch and Warburg [1918] have reported experiments (see Table XIV) from which a similar calculation can be made, as the carbonic acid combination

[1] E. Wichmann [*Pflüger's Arch.* 1921, **189**, p. 108] has likewise shown that Falta and Richter-Quittner's view regarding the difference of hirudin blood is incorrect.

curve of the blood was determined (which had not been previously reported) simultaneously with the experiment with the ox blood which combined with 13·4 vols. % O_2. The values obtained in this experiment are very uncertain because the blood contained few blood corpuscles and there were also local difficulties with the centrifuging. The other experiment of Hasselbalch and Warburg which allows of the calculation being made should give much better results. I have also carried out a few experiments with defibrinated ox blood which will be found in Table XVII.

In calculating D from ox blood experiments it is always assumed that 100 vols. blood corpuscles combine with 45 vols. O_2.

I have performed a number of experiments with defibrinated horse blood which appear in Tables XVIII and XIX. The details of the *technique* were as follows: 50–70 cc. blood were saturated for one hour in the saturator shown in Fig. 3. Instead of the electrode shown in the diagram, a glass cylinder with a rubber cork bored with two holes was substituted through which a long and a short glass tube were introduced. The long tube could be pushed up and down through the cork, the movement being airtight. The gas was conducted by the rubber tube through the glass cylinder before reaching the saturator.

After the completion of saturation a sample of blood was first taken for testing (B_I), whereupon the T tap was turned to position I and the exit rubber tube was removed from its glass tube. The cylinder was then filled with the blood through the glass tube by siphon action avoiding contact with the air. The position of the tap was finally as shown in position II.

When the blood corpuscles had settled a sample was taken through the long glass tube with the help of a rubber tube for pumping out of the serum (S_I) and then another of the blood corpuscle emulsion (C_I). A portion of the serum (S_{II}) was then saturated again and also a portion of blood corpuscles (C_{II}) in a small saturator (Fig. 5) and their gas content estimated in the usual way. Lastly a sample of blood was saturated afresh which before the beginning of the main experiment was saturated with the gas mixture and had been standing in the thermostat (at room temperature), closed in a small saturator, and the sample was pumped out and analysed (B_{II}).

At higher CO_2 tensions the other gas in the spirometer was O_2.

Haematocrite estimations were performed on B_I, C_I and B_{II} as a rule, usually two at a time, and centrifuging was continued until the column of corpuscles was transparent. In those cases where haematocrite estimations were not done the volume of the blood corpuscles was calculated from the amount of oxygen, which is duly noted in the tables.

In the experiments in which solutions rich in blood corpuscles were employed these were the only ones used for the calculation of D, other experiments being used as controls. The values for the oxygen combining power of the corpuscles given in Fig. 9 are all derived from experiments with blood cell emulsions.

Most of the horse blood came from the cattle market. The blood for

experiments 1–3 was fresh and obtained from the serum laboratory of "Landbohöjskolen."

A complete experiment lasted 4–5 hours. Saturation was usually finished after 3½–4 hours.

In experiments with ox blood it was passed from the saturator into a centrifuge tube, the rubber tube being put right down to the bottom. The blood then rose slowly in the tube which was closed immediately with a cork, and melted paraffin poured over it. After centrifuging the serum and blood cell emulsion were resaturated separately.

Hasselbalch [1916, 2] has carried out the determinations[1] of $p\lambda'_{(s)}$ given in Table XII. They were done with the technique of Hasselbalch and the remarks previously made about this technique refer also to these experiments. It should be stated however that the oxygen error is much less in these experiments than with blood.

In Table XIII a number of determinations of $p\lambda'_{(s)}$ are given which were carried out with the small saturator electrode in the usual way. The oxygen in the spirometer gas was always estimated but on no occasion did it exceed 1 mm.; as a rule it was about 0·5 mm.

From the mean of Hasselbalch's four experiments at 38°

$$p\lambda'_{(s)} = 6{\cdot}13 \text{ and consequently } p\lambda_{(s)} = 6{\cdot}14.$$

From the 23 estimations given in the table I get at 38°

$$p\lambda'_{(s)} = 6{\cdot}144 \pm 0{\cdot}0042.$$

The standard error was 0·018. From the mean of 19 estimations at 18°

$$p\lambda'_{(s)} = 6{\cdot}283 \pm 0{\cdot}0042.$$

The standard error was 0·019. From these we get at 38°

$$p\lambda_{(s)} = 6{\cdot}155 \pm 0{\cdot}0042,$$

and at 18°

$$p\lambda_{(s)} = 6{\cdot}294 \pm 0{\cdot}0042.$$

The tables show that there is no variation of the constants determined by the reaction, which is in harmony with the small standard deviation.

If the estimations at room temperature and at 38° are combined we get

$$p\lambda_{(s)}t^\circ = 6{\cdot}294 + (18^\circ - t^\circ)\,0{\cdot}007. \quad \ldots\ldots\ldots\ldots\ldots(131)$$

In Fig. 6 the values of D are plotted as ordinates and the apparent hydrogen ion activity exponents as abscissae. The individual determinations, as will be seen, are well distributed about the curve and it will be noted D varies considerably in the range of reaction dealt with. The cause of this variation will be the subject of a thorough inquiry in the last chapter.

Some points on the figure are marked with a circle. They refer to the determinations in Table XIX. It will be observed that the addition of a solution isotonic with the blood, 5 cc.–100 cc. of 0·9 % NaCl solution (0·154n), does not change D, while a similar quantity of a hypertonic salt solution, 0·5n $NaHCO_3$ and 1·0n NaCl, makes D smaller. This last fact is of par-

[1] $p\lambda'_{(s)}$ has been formed analogically with $p\Lambda'_{(s)}$ in equation (127).

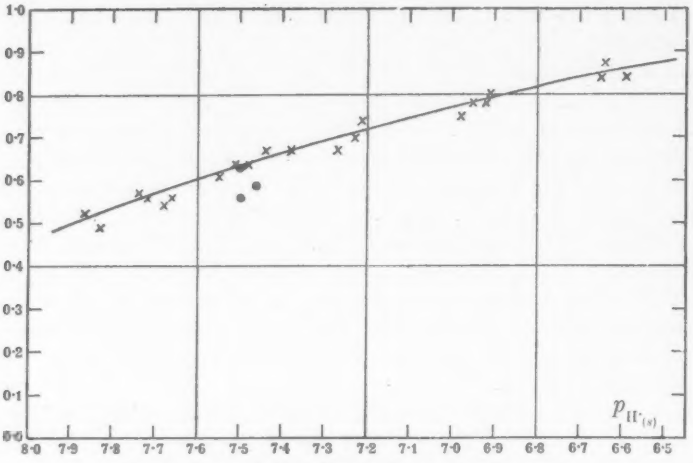


Fig. 6. Horse blood (defibrinated), room temperature.

$$\frac{\text{cc. combined } CO_2 \text{ in 100 cc. blood corpuscles}}{\text{cc. combined } CO_2 \text{ in 100 cc. serum}} = D.$$

ticular interest and is in harmony with what would be expected from theoretical considerations.

In the experiment with the addition of physiological salt solution the blood combines with 62·5 vols. % CO_2 at a CO_2 tension of 37·3 mm., with a serum of $p_{H'}$ 7·50, while in the corresponding serum 73·5 vols. % CO_2 (combined) are found. In the experiment with hypertonic salt solution the blood combines with the same amount of CO_2 (62·7 vols. %) at practically the same tension (38·5 mm.) and the same $p_{H'}$ (7·50), but the serum combines with *rather more* CO_2 (76·0 vols. %).

The CO_2 combined by the serum is 4 % greater in the last experiment than in the first. At the same time it can be demonstrated that the volume of the blood corpuscles has diminished by 12 %.

Table XII. $p\lambda'_{(s)}$ calculated from K. A. Hasselbalch's experiments, *Biochem. Zeitsch.* **78**, p. 123.

	mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(s)}$	$p\lambda'_{(s)}$	Corrected $p_{H'(s)}$	Corrected $p\lambda'(s)$
38°. Ox serum III	41·8	62·0	7·44	6·12	7·45	6·13
	14·5	54·3	7·83	6·11	7·83	6·11
38°. Ox serum IV	40·6	60·9	7·45	6·13	7·46	6·14
	59·8	59·8	7·45	6·12	7·46	6·13
						Mean 6·13
18°.	41·0	64·6	7·36	6·24	7·37	6·25

Table XIII. Determinations of $p\lambda'_{(s)}$ in serum carried out with the small saturator electrode.

Temp.	mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(s)}$	$p\lambda'_{(s)}$ 18°	$p\lambda'_{(s)}$ 38°	
18·5	512·5	71·1	6·31	6·24	—	Series A
18·5	119·6	64·4	—	—	—	1st day
18·5	18·7	58·2	—	—	—	
19·5	41·2	62·6	7·36	6·24	—	Horse blood
19·5	12·7	55·8	7·82	6·24	—	2nd day
18	545·9	75·9	6·32	6·21	—	Series A
18·5	118·6	69·4	6·95	6·26	—	Ox serum
19	43·1	64·7	7·36	6·24	—	
19	16·0	58·5	7·71	6·22	—	
			Mean	6·23		
38	550·1	69·1	6·43	—	6·18	Series B
20	573·7	71·4	6·28	6·24	—	1st day
38	79·6	—	7·19	—	—	
19·5	83·1	65·3	7·12	6·28	—	Horse serum
38	12·9	50·7	7·89	—	6·14	2nd day
19·5	13·1	55·6	7·85	6·28	—	
38	32·6	58·1	7·50	—	6·10	
19·5	34·1	61·1	7·49	6·29	—	
38	521·7	75·5	—	—	—	Series B
18	546·8	65·1	6·37	6·30	—	Ox serum
38	79·8	68·1	7·24	—	6·16	
18	83·6	76·1	7·16	6·28	—	
21	595·0	75·4	—	—	—	Series B
21	122·9	71·9	—	—	—	1st day
21	40·6	65·3	7·46	6·31	—	Ox serum
21·5	16·1	61·8	7·80	6·28	—	3rd day
21·5	40·5	66·0	7·40	6·25	—	
21·5	70·2	68·5	7·22	6·29	—	
21	558·5	79·5	6·38	6·30	—	Series B
21	96·7	74·4	7·12	6·30	—	Horse serum
21	14·2	63·0	7·86	6·28	—	
22	18·9	65·6	6·75	6·25	—	Series B
21·5	54·3	67·6	7·32	6·29	—	Horse serum
21·5	38·4	67·5	7·48	6·29	—	
19·5	96·3	74·9	7·12	6·30	—	Series B
19	15·4	63·5	7·82	6·28	—	Horse serum
19	9·3	61·8	8·03	6·28	—	
			Mean	6·283		
38	24·6	63·4	7·70	—	6·13	Series B
38	23·5	63·8	7·72	—	6·14	Horse serum
38	45·6	67·5	7·45	—	6·15	
38	104·6	71·8	7·14	—	6·16	
38	430·3	77·3	6·56	—	6·15	
38	327·4	71·2	6·64	—	6·15	Series B
38	69·6	61·9	7·24	—	6·15	Ox serum
38	535·6	74·9	6·43	—	6·14	Series B
38	130·7	69·7	7·02	—	6·14	1st day
38	35·2	63·3	7·54	—	6·13	Horse serum
38	10·4	58·1	8·01	—	6·12	3rd day
38	106·7	69·2	7·11	—	6·15	
38	510·7	80·1		—	—	Series B
38	103·7	74·7	7·15	—	6·13	Ox serum
38	22·7	62·6	7·74	—	6·16	
38	59·5	69·0	7·34	—	6·14	
38	546·7	78·5	—	—	—	Series B
38	53·8	67·8	7·40	—	6·16	Ox serum
38	562·1	78·7	6·44	—	6·15	Series B
38	84·5	68·7	7·23	—	6·18	Human serum. (Patient with chronic nephritis)
38	15·2	57·2	—	—	—	
38	18·3	60·7	7·81	—	6·14	
				Mean	6·144	

Table XIV. The value D is calculated from Hasselbalch and Warburg's experiments 4 and 6. *Biochem. Zeitsch.* **86**, p. 417. Defibrinated ox blood.

In experiment 4 the volume of the blood cells is estimated at 27·5 % as the blood combined with 12·4 vols. % O_2.

In experiment 6 the volume of the blood cells is estimated at 68 % in the blood cell suspension B; the suspension combined with 31·3 vols. % O_2.

The values for the corresponding carbonic acid combinations in serum and blood cells are determined by interpolation.

Experiment 4.

Blood		Serum		
mm. Hg CO_2	Vols. % CO_2 combined	mm. Hg CO_2	Vols. % CO_2 combined	mm. Hg CO_2 during centrifuging
12·5	43·3	110·4	62·3	12·7
		193·9	64·4	
12·7	44·5			
46·5	61·1			
58·8	65·7	25·0	61·8	58·8
94·1	69·7	96·8	69·9	
181·9	80·0	173·9	74·0	
185·4	79·7			
		29·0	67·3	89·7
377·5	90·2	101·1	75·4	
		182·9	76·3	
		23·4	68·5	181·9
		134·9	79·3	
		401·4	87·7	
430·5	88·0	17·9	75·4	430·5
		160·0	88·2	
		737·0	91·6	
737·5	99·3	26·4	80·6	737·0
		161·1	92·6	
		428·3	95·0	

mm. Hg CO_2	Vols. % combined CO_2 in serum	Vols. % combined CO_2 in blood cells	D	p_H in serum
12·7	52	23	0·35	7·78
58·8	66	66	1·00	7·22
89·7	74	52	0·70	7·09
181·9	82	65	0·80	6·83
430·5	90	75	0·87	6·49
737·0	96	107	1·11	6·29

Experiment 6.

Serum B. CO_2 tension during centrifuging, 65 mm.		Blood cell suspension B	
mm. Hg CO_2	Vols. % CO_2 combined	mm. Hg CO_2	Vols. % CO_2 combined
19·5	61·6	29·8	46·1
79·8	69·6	86·8	67·7
182·0	73·0	159·8	81·2
378·3	73·7	387·7	100·8

from which at 65·0 mm. Hg CO_2 in serum 68 vols. %, in blood cells 55·9, $D = 0·82$, p_H in serum = 7·23.

Table XV. The value D calculated from J. Joffe and E. P. Poulton's experiments with human blood at 38°. *J. Physiol.* **54**, pp. 148–149, Tables II, III and V.

	Oxygenated blood Vols. % combined CO_2				Reduced blood Vols. % combined CO_2				
mm. Hg CO_2	in serum	in blood cells	D	p_H calc. in serum	in serum	in blood cells	D	p_H calc. in serum	
10	29·2	14·5	0·50	7·77	31·3	21·5	0·69	7·81	
20	38·0	23·3	0·61	7·59	42·1	30·2	0·72	7·64	J.J.'s defibrinated blood
30	44·8	29·6	0·66	7·49	48·7	36·7	0·75	7·52	
40	50·1	35·1	0·70	7·41	54·4	41·6	0·77	7·45	
55	57·3	40·7	0·71	7·31	61·4	48·1	0·78	7·36	Blood cell volume 50·93
70	63·1	45·4	0·72	7·27	67·3	54·2	0·81	7·30	
90	68·0	49·5	0·73	7·19	73·4	61·4	0·84	7·23	
156·0	77·2	58·3	0·76	7·01	—	—	—	—	Blood cell volume 53·5
180·0	81·5	57·2	0·70	6·97	—	—	—	—	
110·3	73·8	54·2	0·74	7·14	—	—	—	—	
157·0	76·4	65·1	0·85	7·00	—	—	—	—	
376·3	97·5	83·6	0·85	6·72	—	—	—	—	
610·0	108·9	92·9	0·85	6·56	—	—	—	—	
278·0	97·2	73·9	0·76	6·86	—	—	—	—	
477·0	100·5	91·8	0·91	6·63	—	—	—	—	
40	55·3	25·5	0·46	7·45	60·8	36·0	0·59	7·49	J.J.'s oxalated blood
55	65·3	25·7	0·39	7·38	67·8	42·5	0·63	7·40	Blood cell vol. 44·5
15	36·6	22·7	0·62	7·70	40·8	20·6	0·51	7·75	W.R.'s oxalated
25	45·8	24·3	0·53	7·58	49·9	31·8	0·64	7·61	blood
35	52·0	29·6	0·57	7·48	55·9	40·7	0·73	7·51	Blood cell vol. 39·77
45	58·2	33·0	0·57	7·42	59·8	44·5	0·74	7·44	
60	62·2	40·6	0·65	7·32	63·8	45·4	0·71	7·34	
75	63·8	42·4	0·66	7·23	65·0	47·9	0·74	7·25	

Table XVI. The value of D calculated from L. S. Fridericia's experiment 7. *J. Biol. Chem.* **42**, p. 254. Ox blood with 0·1 % sodium oxalate. 38 vols. % blood cells at 17°.

mm. Hg	Vols. % CO_2 combined in plasma	Vols. % CO_2 combined in blood cells	D (HCO'_3)	p_H (calc.) in plasma	Gram NaCl in 100 cc. plasma	Gram NaCl in 100 cc. blood cells	D (Cl')
0·08	23·4	16·6	0·71	9·46	0·578	0·260	0·45
6·1	42·6	27·6	0·65	8·04	0·533	0·334	0·63
39·1	67·6	62·0	0·92	7·44	0·511	0·369	0·72

Table XVII. The value of D in ox blood. 100 cc. ox blood cells combined with 45 cc. O_2.

Temp.	mm. Hg CO_2	Vols. % combined CO_2	(p_H) (calc.)	Vols. % combined O_2	
18	35·5	64·0	7·46	—	Serum
		58·6	—	16·0	Blood haematocrite determination 35
		50·7	—	35·8	Blood cell suspension
					from serum and blood ... ... D=0·79
					from serum and blood cell suspension D=0·74
18	43·3	74·4	7·44	—	Serum
		69·1	—	10·9	Blood
		61·6	—	29·6	Blood cell suspension
					from serum and blood ... ... D=0·79
					from serum and blood cell suspension D=0·74
18	21·9	63·5	7·67	—	Serum
		60·2	—	10·9	Blood
		48·9	—	33·5	Blood cell suspension
					from serum and blood ... ... D=0·78
					from serum and blood cell suspension D=0·69
18	9·8	56·8	7·97	—	Serum
		50·2	—	10·9	Blood
					from serum and blood ... ... D=0·52
18	22·5	62·5	7·65	—	Serum
		56·9	—	15·7	Blood
		49·2	—	24·5	Blood cell suspension
					from serum and blood ... ... D=0·73
					from serum and blood cell suspension D=0·75

Table XVIII. The value of D in defibrinated horse blood at room temperature.

Temp.	mm. Hg CO_2	Vols. % combined CO_2	Mean of p_H calc. in serum	Vols. % combined O_2	Haematocrite number	Vols. % combined O_2 in blood cells			Vols. % combined CO_2 in blood cells	D
19	34·7	59·3		20·3	41·5	48·9	B_I	from B_I, S_I and haematocrite B_I	44·4	
		69·9		0·2	—	—	S_I			
		51·1		36·0	—	—	C_I	„ C_I, S_I „ B_I	44·4	
		68·7		0·9	—	—	S_{II}			
			7·51							*0·64*
17	15·4	46·2		20·2	40·0	50·5	B_I	„ B_I, S_I „ B_I	30·0	
		57·0		0·6	—	—	S_I			
		40·4		35·1	—	—	C_I	„ C_I, S_I „ B_I	33·1	
		56·1		0·6	—	—	S_{II}			
			7·76							*0·57*
18	82·2	76·1		20·0	41·0	48·9	B_I	„ B_I, S_I „ B_I	61·7	
		87·1		1·3	—	—	S_I			
		69·2		34·7	—	—	C_I	„ C_I, S_I „ B_I	61·1	
		69·4		33·2	66·5	*49·9*	C_{II}			
		87·2		1·6	—	—	S_{II}	„ C_{II}, S_{II} „ C_{II}	60·8	
		76·9		19·9	—	—	B_{II}	„ B_{II}, S_{II} „ B_I	62·2	
			7·23							*0·70*
18	38·4	59·3		19·8	40·4	49·0	B_I	„ B_I, S_I „ B_I	37·2	
		74·1		0·5	—	—	S_I			
		55·1		35·8	—	—	C_I	„ C_I, S_I „ B_I	48·1	
		53·5		34·1	66·0	*51·8*	C_{II}			
		71·8		0·7	—	—	S_{II}	„ C_{II}, S_{II} „ C_{II}	45·2	
		60·1		19·2	37·5	51·1	B_{II}	„ B_{II}, S_{II} „ B_{II}	40·7	
			7·48							*0·64*
17	17·3	47·4		19·7	40·3	49·2	B_I	„ B_I, S_I „ B_I	32·3	
		57·7		0·4	—	—	S_I			
		39·7		37·9	—	—	C_I	„ C_I, S_I „ B_I	34·3	
		39·1		36·2	65·8	(55·0)	C_{II}			
		56·4		0·8	—	—	S_{II}	„ C_{II}, S_{II} „ B_I	29·9	
		47·0		18·8	39·5	47·6	B_{II}	„ B_{II}, S_{II} „ B_{II}	32·7	
			7·72							*0·56*
17	86·2	78·4		19·8	41·4	48·5	B_I	„ B_I, S_I „ B_I	63·8	
		88·9		1·1	—	—	S_I			
		71·7		35·5	—	—	C_I	„ C_I, S_I „ B_I	65·4	
		89·0		1·1	—	—	S_{II}			
		74·8		31·3	—	—	C_{II}	„ C_{II}, S_{II} „ B_I	65·8	
		78·1		18·6	36·5	51·0	B_{II}	„ B_{II}, S_{II} „ B_{II}	59·2	
			7·21							*0·74*
19	178·3	90·4		18·4	41·3	44·6	B_I	„ B_I, S_I „ B_I	78·0	
		99·1		0·8	—	—	S_I			
		81·9		35·7	—	—	C_I	„ C_I, S_I „ B_I	77·6	
		82·2		35·0	75·5	*46·6*	C_{II}			
		98·7		1·2	—	—	S_{II}	„ C_{II}, S_{II} „ C_{II}	76·9	
		89·1		17·9	39·6	45·3	B_{II}	„ B_{II}, S_{II} „ B_{II}	74·5	
			6·95							*0·78*
18	182·7	95·1		23·8	48·9	48·7	B_I	„ B_I, S_I „ B_I	80·4	
		109·3		1·4	—	—	S_I			
		88·5		36·6	—	—	C_I	„ C_I, S_I „ C_{II}	81·8	
		88·1		36·6	77·4	*47·3*	C_{II}			
		109·6		0·8	—	—	S_{II}	„ C_{II}, S_{II} „ C_{II}	82·4	
		96·4		22·7	48·8	46·5	B_{II}	„ B_{II}, S_{II} „ B_{II}	82·6	
			6·98							*0·75*
18	76·0	75·1		23·0	47·5	48·4	B_I	„ B_I, S_I „ B_I	57·9	
		90·7		1·0	—	—	S_I			
		68·6		36·0	—	—	C_I	„ C_I, S_I „ C_{II}	61·2	
		67·1		36·6	76·3	*48·0*	C_{II}			
		89·6		0·5	—	—	S_{II}	„ C_{II}, S_{II} „ C_{II}	60·2	
		75·1		22·7	47·0	48·3	B_{II}	„ B_{II}, S_{II} „ B_{II}	58·7	
			7·28							*0·67*

Table XVIII (*continued*)

Temp.	mm. Hg CO_2	Vols. % combined CO_2	Mean of p_H calc. in serum	Vols. % combined O_2	Haematocrite number	Vols. % combined O_2 in blood cells				Vols. % combined CO_2 in blood cells	D
18	35·5	66·7		—	39·0	—	B_I	from B_I, S_I and haematocrite		B_I 46·6	
		79·5		0·5	—	—	S_I				
		55·5		37·6	—	—	C_I	„ C_I, S_I	„	C_{II} 48·4	
		53·8		38·8	79·7	48·7	C_{II}				
		78·7		0·4	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 47·7	
		66·7		—	38·3	—	B_{II}	„ B_{II}, S_{II}	„	B_{II} 47·3	
			7·55								*0·61*
18	461·2	115·7		19·0	—	—	B_I	„ B_I, S_I	„	C_{II} 98·2	
		128·2		0·4	—	—	S_I				
		113·8		34·7	—	—	C_I	„ C_I, S_I	„	C_{II} 109·3	
		110·1		35·3	77·5	*45·6*	C_{II}				
		126·5		0·7	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 105·3	
		117·6		18·7	41·8	44·8	B_{II}	„ B_{II}, S_{II}	„	B_{II} 105·2	
			6·65								*0·84*
17	12·6	60·5		0·4	—	—	S_I	„ C_I, S_I	„	C_{II} 32·9	
		40·1		36·9	—	—	C_I				
		35·8		39·4	79·1	*49·8*	C_{II}	„ C_{II}, S_{II}	„	C_{II} 29·4	
		60·0		0·4	—	—	S_{II}				
		49·0		18·5	37·4	49·5	B_{II}	„ B_{II}, S_{II}	„	B_{II} 30·5	
			7·87								*0·52*
18	443·1	99·5		13·4	30·4	44·1	B_I	„ B_I, S_I	„	B_I 78·6	
		97·6		33·2	—	—	C_I				
		108·7		0·2	—	—	S_I	„ C_I, S_I	„	C_{II} 93·8	
		90·9		32·6	73·0	*44·7*	C_{II}				
		104·7		0·4	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 85·8	
		98·5		13·6	31·0	43·9	B_{II}	„ B_{II}, S_{II}	„	B_{II} 84·4	
			6·59								*0·84*
19	21·5	52·7		13·7	27·9	49·1	B_I	„ B_I, S_I	„	B_I 32·4	
		60·7		0·2	—	—	S_I				
		40·6		38·5	—	—	C_I	„ C_I, S_I	„	C_{II} 34·9	
		38·4		38·8	78·3	*49·6*	C_{II}				
		59·8		0·7	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 32·5	
		52·6		13·1	26·5	49·4	B_{II}	„ B_{II}, S_{II}	„	B_{II} 32·8	
			7·66								*0·56*
18	413·9	108·1		18·2	41·3	44·2	B_I	„ B_I, S_I	„	B_I 98·4	
		114·9		0·4	—	—	S_I				
		103·3		33·9	—	—	C_I	„ C_I, S_I	„	B_I 99·8	
		101·2		33·2	75·0	44·3	C_{II}				
		111·8		0·7	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 97·6	
			6·64								*0·87*
18	14·3	49·2		18·9	37·8	50·1	B_I	„ B_I, S_I	„	B_I 26·8	
		63·2		0·6	—	—	S_I				
		38·7		38·7	—	—	C_I	„ C_I, S_I	„	B_I 30·0	
		37·7		38·2	76·0	*50·2*	C_{II}				
		58·9		0·5	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 29·7	
		48·7		17·5	36·8	47·5	B_{II}	„ B_{II}, S_{II}	„	B_{II} 31·3	
			7·83								*0·49*
18	217·3	99·4		18·8	40·8	46·1	B_I	„ B_I, S_I	„	B_I 81·1	
		111·9		0·5	—	—	S_I				
		94·2		35·8	—	—	C_I	„ C_I, S_I	„	C_{II} 89·1	
		92·5		35·3	76·5	*46·1*	C_{II}				
		110·1		0·5	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 88·5	
		99·3		18·5	39·9	47·9	B_{II}	„ B_{II}, S_{II}	„	B_{II} 83·3	
			6·91								*0·80*
17	19·4	51·4		20·8	41·5	50·3	B_I	„ B_I, S_I	„	B_I 39·6	
		59·8		0·9	—	—	S_I				
		40·0		38·9	—	—	C_I	„ C_I, S_I	„	C_{II} 32·7	
		41·8		38·1	75·5	*50·4*	C_{II}				
		59·3		0·5	—	—	S_{II}	„ C_{II}, S_{II}	„	C_{II} 31·3	
		50·2		19·9	40·0	49·7	B_{II}	„ B_{II}, S_{II}	„	B_{II} 36·0	
			7·68								*0·54*

Table XVIII (*continued*)

Temp.	mm. Hg CO_2	Vols. % combined CO_2	Mean of p_H calc. in serum	Vols. % combined O_2	Haematocrite number	Vols. % combined O_2 in blood cells						Vols. % combined CO_2 in blood cells	D
17	45·1	67·2		20·6	41·8	49·4	B_I	from B_I,	S_I	and haematocrite	B_I	50·4	
		79·2		0·6	—	—	S_I						
		58·3		37·7	—	—	C_I	„ C_I,	S_I	„	C_{II}	52·1	
		59·9		35·9	72·9	*49·3*	C_{II}						
		78·1		0·9	—	—	S_{II}	„ C_{II},	S_{II}	„	C_{II}	53·1	
		67·1		19·9	—	—	B_{II}	„ B_{II},	S_{II}	„	C_{II}	52·0	
			7·44										*0·67*
19	42·3	53·6		21·6	45·0	47·9	B_I	„ B_I,	S_I	„	B_I	42·7	
		62·6		0·3	—	—	S_I						
		46·4		37·7	—	—	C_I	„ C_I,	S_I	„	C_{II}	42·0	
		62·3		0·7	—	—	S_{II}						
		46·6		36·0	75·0	*48·0*	C_{II}	„ C_{II},	S_{II}	„	C_{II}	41·3	
		52·9		20·7	43·4	47·7	B_{II}	„ B_{II},	S_{II}	„	B_{II}	40·3	
			7·38										*0·67*
19	172·4	89·0		0·7	—	—	S_I	„ C_I,	S_I	„	C_{II}	72·0	
		75·7		35·8	—	—	C_I						
		73·2		35·3	77·0	*47·1*	C_{II}	„ C_{II},	S_{II}	„	C_{II}	68·2	
		90·8		1·0	—	—	S_{II}						
			6·92										*0·78*

Table XIX. The value of D in defibrinated horse blood with various additions.

Temp.	mm. Hg CO_2	Vols. % combined CO_2	Mean of p_H calc. in serum	Vols. % combined O_2	Haematocrite number	Vols. % combined O_2 in blood cells						Vols. % combined CO_2 in blood cells	D
				100 cc. blood + 5 cc. 0·9 % NaCl sol. (isotonic with blood)									
18	37·3	62·5		19·6	40·3	48·6	B_I	from B_I,	S_I	and haematocrite	B_I	46·1	
		73·5		0·4	—	—	S_I						
		53·3		36·6	—	—	C_I	„ C_I,	S_I	„	C_{II}	47·8	
		51·3		37·3	75·8	*49·2*	C_{II}						
		72·7		0·2	—	—	S_{II}	„ C_{II},	S_{II}	„	C_{II}	44·5	
		61·4		18·4	38·8	47·4	B_{II}	„ B_{II},	S_{II}	„	B_{II}	43·4	
			7·50										*0·63*
				100 cc. blood + 5 cc. $n/2$ $NaHCO_3$ (hypertonic to blood)									
19	77·6	118·5		18·6	36·0	51·7	B_I	from B_I,	S_I	and haematocrite	B_I	83·0	
		138·5		0·3	—	—	S_I						
		97·4		37·1	—	—	C_I	„ C_I,	S_I	„	C_{II}	81·0	
		96·6		37·5	72·4	*51·8*	C_{II}						
		136·7		0·7	—	—	S_{II}	„ C_{II},	S_{II}	„	C_{II}	81·2	
		119·3		18·9	36·0	52·5	B_{II}	„ B_{II},	S_{II}	„	B_{II}	88·6	
			7·46										*0·59*
				100 cc. blood + 5 cc. $n/1$ NaCl (more hypertonic)									
19	38·5	62·7		19·1	34·0	56·2	B_I	from B_I,	S_I	and haematocrite	B_I	36·8	
		76·0		0·2	—	—	S_I						
		56·2		35·4	—	—	C_I	„ C_I,	S_I	„	C_{II}	44·7	
		56·5		30·6	*54·5*	*56·1*	C_{II}						
		75·3		0·4	—	—	S_{II}	„ C_{II},	S_{II}	„	C_{II}	40·7	
			7·50										*0·56*

The curve with a continuous line in Fig. 7 is the same as that in Fig. 6 (horse blood at room temperature); the "recumbent" crosses represent the values of D in oxygenated blood calculated from the experiments of Joffe

and Poulton [1920] with defibrinated human blood at 38°; the "erect" crosses represent the values of D in almost completely reduced blood; the marks enclosed in squares refer to oxalated blood.

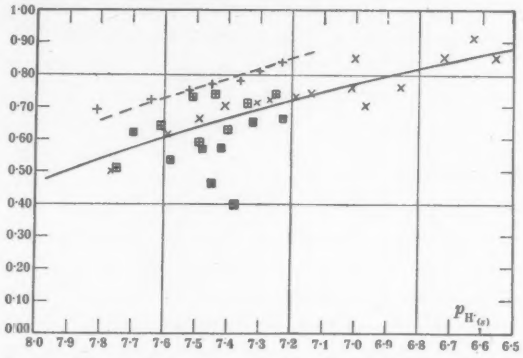


Fig. 7. Joffe and Poulton's results compared with experiments with horse blood.

——— Oxygenated horse blood at room temperature, defibrinated.
— — Reduced human blood at 38°, defibrinated.
× Defibrinated human blood, oxygenated
\+ ,, ,, reduced
⊠ Oxalated ,, oxygenated
⊞ ,, ,, reduced
} 38°. The value of D.

Joffe and Poulton's experiments with defibrinated human blood at 38° give values for D which, allowing for experimental error, are identical with those for horse blood cited above, while reduced blood gives values which are fairly accurately represented by a curve situated 0·125 higher in the diagram and which runs parallel with the curve for horse blood. The experiments with oxalated blood all give smaller values for D than corresponding ones with defibrinated blood but the values are more irregular. Apart from this the experiments with oxalated blood agree with those with defibrinated blood as regards all the other factors.

The addition of oxalate therefore causes a change in the distribution of carbonic acid in the blood and it is quite analogous to the alteration which takes place in horse blood on the addition of hypertonic salt solution; sodium oxalate in point of fact introduces fresh cations into the plasma which makes the serum hypertonic and causes the blood corpuscles to shrink.

Referring to J. Joffe's experiments on human blood[1] we find at a pressure of 40 mm. CO_2, 45·0 vols. % CO_2 (total) in defibrinated oxygenated blood and 44·5 vols. % in oxalated blood, while in the corresponding serum there are 52·8 vols. % and in plasma 58·0. As regards reduced blood defibrinated contains 50·4 vols. % total CO_2 and oxalated 52·3, while there are 57·1 vols. % in serum against 63·5 vols. % in plasma. At 55 mm. CO_2 exactly similar conditions prevail. It is seen therefore that the CO_2 content of plasma is

[1] J. Joffe and E. P. Poulton [1920, p. 148, Table II].

10 % higher than that of serum and the blood corpuscles have shrunk about 13 %.

Similar conditions would presumably be found in osmotically hypertonic blood (nephritis and sweating baths, diarrhoea, etc.).

D. D. van Slyke and G. E. Cullen [1917] some years ago introduced a method for determining the amount of combined bicarbonate in the blood which is in many ways extremely useful and quite accurate. This method appears to give more variable values for the combined CO_2 than might have been expected from the experiments of J. Christiansen, Douglas and Haldane [1914], Hasselbalch [1916, 2], Donegan and Parsons [1919] and Jarlöv [1919] on the CO_2 combining capacity of whole blood when it is assumed the partition between plasma and blood corpuscles is constant at the same reaction.

In the previously mentioned paper Hasselbalch and Warburg [1918] have cast suspicion on methods based upon the determination of the combined CO_2 in the serum (or plasma) when it is desired to draw conclusions about the "alkali reserve" (van Slyke) or the "reduced hydrogen number" [Hasselbalch, 1916, 1], having pointed out that the Schmidt-Zuntz phenomenon may play some part, provided the blood is not centrifuged at the alveolar CO_2 tension. Recently Joffe and Poulton [1920] in their much cited work have dealt with the same question and put forward a similar explanation of the degree of the dispersion in the experiments of van Slyke and his collaborators.

It has for long seemed strange to the author that the "fortuitous" variations which are associated with van Slyke's method appear to be considerably greater than those we should expect to arise from the Schmidt-Zuntz phenomenon. I think I have already made it clear that the most important cause of the variations is to be sought in the addition of varying amounts of oxalate to the blood before centrifuging it. Van Slyke and Cullen [1917] have shown that oxalate mixed with serum does not alter its combining power with CO_2 but they have overlooked the fact, as far as I am aware, that the oxalate could change the distribution of CO_2 in the blood. Van Slyke and Cullen recommend the addition of 0·5 % oxalate, which is a rather large amount. I have little doubt it would be an advantage to the method if the amount of oxalate employed was always the same and was as small as possible because there will always be a slight want of uniformity on account of the variation in the quantity of blood corpuscles in different experiments.

In the final chapter it will be shown that the Cl' partition between blood corpuscles and serum follows exactly similar laws to that of HCO'_3. It is thus important in determining the amount of Cl' in plasma to pay attention to the amount of oxalate added (the same applies also to fluoride and citrate), just as we should naturally work with a known CO_2 tension as has been particularly pointed out by H. J. Hamburger [1902] and L. S. Fridericia [1920].

After this digression let us revert to the curves. In Fig. 8 the value of D will be found in oxygenated ox blood at room temperature calculated from Fridericia's [1920], Hasselbalch and Warburg's [1918] and my later experi-

ments. The values emanating from the previously mentioned unreliable experiments of Hasselbalch and Warburg are put in parentheses. The other experiments fall evenly on a curve similar to that for reduced human blood according to Joffe and Poulton's [1920] experiments. The experiments are too few to bear further investigation.

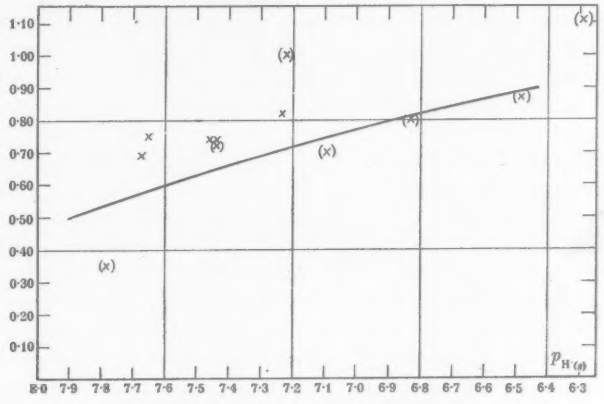


Fig. 8. *D* in ox blood at room temperature.

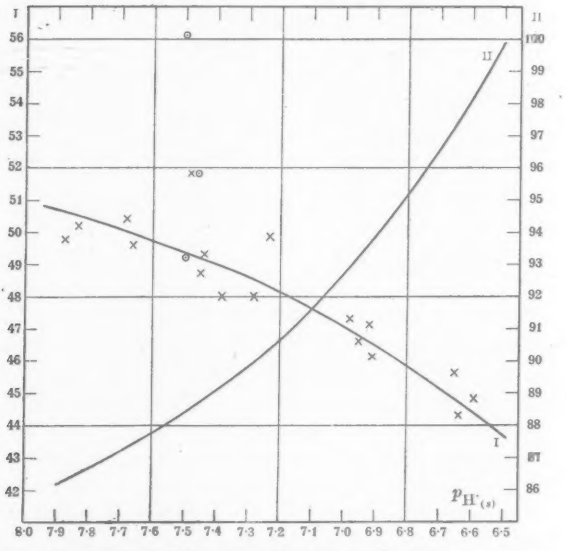


Fig. 9. Curve I. cc. of O_2 in 100 cc. horse blood corpuscles.
„ II. The volume of blood corpuscles as a % of their volume at p_H 6·50.

Fig. 9, curve I, and Table XX show how the oxygen in 100 cc. of horse blood corpuscles varies. In the earlier literature we find the statements of H. Nasse [1878] and Hamburger [1902] on the volume of the blood corpuscles at low CO_2 tensions and at tensions about 1 atmosphere. These statements, however important they may have been for the development of our knowledge of the physical chemistry of the blood, are obviously not accurate. Joffe and Poulton have not been able to demonstrate with certainty the change of volume determined by the reaction with greatly varying CO_2 tensions. The variations shown in the diagram are well grouped about the curve with the exception of two points, so that it will have considerable value on account of its novelty. The variations in volume will in chapter XII be the subject of a theoretical investigation. Curve II gives the volume of the blood corpuscles as a percentage of the volume at p_H 6·50.

At p_H 7·40 as will be seen horse blood cells combine with 49 cc. O_2 per 100 cc. blood cells. A. Norgaard and H. C. Gram [1921] found values diverging from mine in an examination of human blood at physiological reaction; as a mean of three determinations I found 48 vols. %. The difference is presumably in the haemoglobin determinations (see chapter XI, p. 285 footnote). With regard to the volume of ox blood corpuscles I have only a very few determinations available, the mean of which gives 45 cc. O_2 in 100 cc. blood corpuscles at "physiological" p_H, but the determination can very well be a couple of vols. % out.

Table XX.

p_H	Vols. % combined O_2 in blood cells	Vol. of blood cells as a % of vol. at p_H 6·50	p_H	Vols. % combined O_2 in blood cells	Vol. of blood cells as a % of vol. at p_H 6·50
6·50	43·7	100·0	7·30	48·6	89·9
6·60	44·5	98·2	7·40	49·0	89·1
6·70	45·2	96·7	7·50	49·4	88·4
6·80	45·9	95·2	7·60	49·8	87·8
6·90	46·6	93·9	7·70	50·1	87·2
7·00	47·2	92·7	7·80	50·4	86·7
7·10	47·7	91·7	7·90	50·7	86·2
7·20	48·2	90·7			

From the curves in Figs. 6 and 9 we can now calculate the $p\Lambda'_{(s)}$ variations for horse blood at room temperature. This is done in Table XXI and the results are graphically represented in Fig. 10. It will be noted the curves also give a measure of the error committed if p_H of the blood is estimated by the Henderson-Hasselbalch equation and $p\lambda'_{(s)}$ used in the calculation. The error would then always be 0·011 greater than the correction in the table. It will also be observed that it is very easy by choosing a suitable constant to get a good result in calculating p_H from the Henderson-Hasselbalch equation, and that a larger (logarithmic) constant should be used in an alkaline reaction than in a more acid reaction. This is in agreement with what we found in the preceding chapters. The rather steep rise of the curves indicates that

Table XXI.

$p_{H'}$	D	Q	$\frac{100-Q(1-D)}{100}$	$-\log\frac{100-Q(1-D)}{100}$	$-\log\frac{100-Q(1-D)}{100}$ $-\log\Phi_a(CO_2)$
	10 vols. % O_2		$\Psi=5{\cdot}9$	$\Phi_a(CO_2)=1{\cdot}06$	$\log\Phi_a(CO_2)=0{\cdot}027$
7·90	0·50	19·7	0·901	0·045	0·018
7·60	0·60	20·1	0·920	0·036	0·009
7·40	0·66	20·4	0·931	0·031	0·004
7·27	0·70	20·7	0·938	0·028	0·001
6·88	0·80	21·5	0·957	0·019	−0·008
6·50	0·88	22·9	0·973	0·011	−0·016
	15 vols. % O_2		$\Psi=8{\cdot}0$	$\Phi_a(CO_2)=1{\cdot}09$	$\log\Phi_a(CO_2)=0{\cdot}036$
7·90	0·50	29·6	0·852	0·069	0·033
7·60	0·60	30·2	0·879	0·056	0·020
7·40	0·66	30·6	0·896	0·048	0·012
7·27	0·70	31·1	0·907	0·042	0·006
6·88	0·80	32·3	0·935	0·029	−0·007
6·50	0·88	34·4	0·959	0·018	−0·018
	20 vols. % O_2		$\Psi=9{\cdot}9$	$\Phi_a(CO_2)=1{\cdot}10$	$\log\Phi_a(CO_2)=0{\cdot}042$
7·90	0·50	39·4	0·803	0·095	0·053
7·60	0·60	40·2	0·839	0·076	0·034
7·40	0·66	40·8	0·862	0·064	0·022
7·27	0·70	41·4	0·876	0·057	0·015
6·88	0·80	43·0	0·914	0·039	−0·003
6·50	0·88	45·8	0·945	0·025	−0·017
	25 vols. % O_2		$\Psi=11$	$\Phi_a(CO_2)=1{\cdot}12$	$\log\Phi_a(CO_2)=0{\cdot}049$
7·90	0·50	49·3	0·753	0·123	0·074
7·60	0·60	50·9	0·796	0·099	0·050
7·40	0·66	51·0	0·827	0·082	0·033
7·27	0·70	51·8	0·845	0·073	0·024
6·88	0·80	53·8	0·893	0·049	0·000
6·50	0·88	57·3	0·931	0·031	−0·018
	30 vols. % O_2		$\Psi=12$	$\Phi_a(CO_2)=1{\cdot}14$	$\log\Phi_a(CO_2)=0{\cdot}057$
7·90	0·50	59·1	0·705	0·152	0·095
7·60	0·60	60·3	0·759	0·120	0·063
7·40	0·66	61·2	0·792	0·101	0·044
7·27	0·70	62·1	0·814	0·089	0·032
6·88	0·80	64·5	0·871	0·060	0·003
6·50	0·88	68·7	0·916	0·038	−0·019

Donegan and Parsons' estimations in marked alkaline reactions are mostly correct.

As already mentioned the curves are so constructed that the correction for defibrinated horse blood can be directly read when the $p_{H'}$ is calculated with (130).

In the calculation of the $p_{H'(s)}$ of human defibrinated oxygenated blood at 38° the curves are similarly directly applicable. (The condition for this should be that D as well as Q is the same in oxygenated human blood at 38° as in oxygenated horse blood at 18°, but the error due to Q being slightly different will be negligible.) In using the correction curves for calculating the $p_{H'(s)}$ value of oxygenated defibrinated ox blood at room temperature and of reduced defibrinated human blood at 38°, one proceeds in such a way that having determined the $p_{H'}$ value by means of (130), the $p_{H'(s)}$ value is looked for on the continuous line curve of Fig. 7, which has the same D value as the calculated $p_{H'(s)}$ on the interrupted line curve, and then the correction corresponding to

the new $p_{H'}$ is read off Fig. 10. This manoeuvre is most easily performed by subtracting 0·40 from the value of $p_{H'}$ calculated with (130) and then using the resulting number for seeking the correction in Fig. 10. The error committed by this method will hardly be appreciable in the second decimal place.

It will be noticed from the course of the correction curves that $p\Lambda'_{(s)}$ increases with the $p_{H'}$ and with increasing amount of haemoglobin in the blood. That this can only just be demonstrated with the potentiometer in

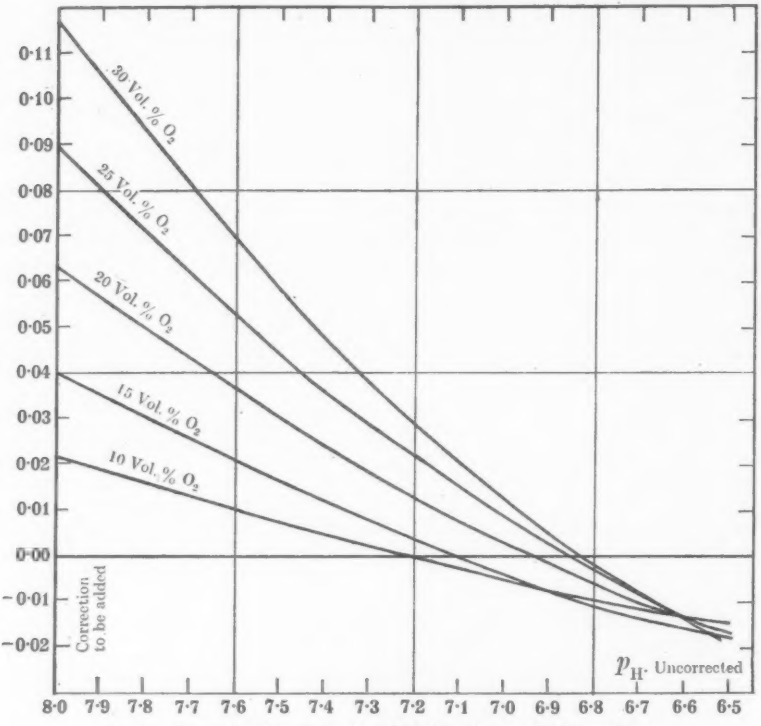


Fig. 10. Correction for use in the calculation of $p_{H'(s)}$ for horse blood at room temperature and human blood at 38°.

the case of the variation determined by the reaction and not at all in the case of that determined by the concentration is due to the fact that potential measurements, even in the excellent arrangement employed by Parsons whose method can hardly be improved upon at present, are not sufficiently reliable in their application to blood. It follows therefore that the $p_{H'}$ value ought to be determined according to the principles given here if the best possible results are desired.

It will also be observed that $p\Lambda'_{(s)}$, when calculated as described in this

chapter, is larger than the constant found in an earlier one. The reason is presumably that the blood corpuscles in spite of all precautions are to a certain extent "deleterious" to the platinum electrode, even in Parsons' and Donegan and Parsons' experiments where saturation and measurement of potential were undertaken in different vessels.

Lastly it is important to note that the calculations of Hasselbalch using his p_{K_1} curve gave much more certain results than by a measurement of potential, and that the results obtained can easily be rectified with the help of the constants given here.

It has often been attempted to estimate the reaction of blood (and serum) colorimetrically and in later years such methods—partly combined with diffusion processes—have been described by Levy, Rowntree and Marriot [1915], W. M. Bayliss [1919] and quite recently in a very refined form by H. H. Dale and C. Lovatt Evans [1920].

J. Lindhard [1921] has devised a micro-method on Dale and Evans' principle which allows of a determination of the $p_{H^{\cdot}}$ value with a relative accuracy of 0·02.

With the method of calculating $p_{H^{\cdot}}$ given here, which is entirely based on a refinement of the Henderson-Hasselbalch principle, the apparent hydrogen ion activity exponent by using the proper method of CO_2 determination can be reckoned with a relative accuracy of 0·005 in any two determinations in human blood at 38° and horse blood at room temperature, and with an error which does not exceed the absolute value by more than 0·015. In human blood and ox blood at room temperature two determinations can be carried out which do not differ by more than about 0·01 relative to each other and with an error which does not exceed the absolute value by more than 0·03.

In conjunction with the above a few examples of the calculation of $p_{H^{\cdot}(s)}$ with Henderson's and Hasselbalch's equation may be given. Let us examine a sample of human blood with 20 vols. % combined O_2 at 38°. The relative absorption coefficient is 0·91. At 10 mm. CO_2 we find 25 vols. % total CO_2.

The amount absorbed is

$$\frac{10 \times 0{\cdot}5554 \times 91}{7{\cdot}60 \times 100} = 0{\cdot}7 \text{ vol. \%}.$$

The amount combined is thus 24·3 vols. %:

$$\log 24{\cdot}3 - \log 0{\cdot}7 = 1{\cdot}563.$$

Converting the values from Hasselbalch's 1916 curve to $p_{H^{\cdot}}$ (Bjerrum) we get

$$p_{H^{\cdot}(s)} = 7{\cdot}727.$$

Using Parsons' and Donegan and Parsons' $p\Lambda'_{(s)}$ we get

$$p_{H^{\cdot}(s)} = 7{\cdot}734.$$

With the author's constant $p_{H^{\cdot}(s)} = 7{\cdot}710$,

and by calculating with (130) and correcting with Fig. 10

$$p_{H^{\cdot}(s)} = 7{\cdot}751.$$

At 40 mm. we find in a similar blood 50 vols. % total CO_2 and consequently 47·3 vols. % combined CO_2, from which, in a similar manner to the previous example, we get

		Warburg	
Hasselbalch	Parsons and Donegan	(126)	(130) and correction
$p_{H'(s)} = 7{\cdot}374$	7·421	7·397	7·418

and for blood at 300 mm. CO_2 with 90 vols. % total CO_2 and 70·1 vols. % combined CO_2

		Warburg	
Hasselbalch	Parsons and Donegan	(126)	(130) and correction
$p_{H'(s)} = 6{\cdot}719$	6·787	6·763	6·750

CHAPTER VII

THE REACTION OF THE BLOOD CORPUSCLES.

Although the majority of workers who have investigated the reaction of the blood have realised that what is generally called "the hydrogen ion concentration of the blood" is really only "the hydrogen ion concentration of the serum," few have attempted to get some knowledge of the reaction in the interior of the blood corpuscles, a question however which is of the greatest interest because analogies with other cells may be drawn from it if similar factors determine the difference in reaction between serum and blood cells, and serum and tissue cells. Hasselbalch and Lundsgaard [1912] and later J. M. de Corral y Garcia [1914] claim to have shown that blood cells at physiological CO_2 tensions are more acid than the corresponding serum. The evidence produced is however faulty, as the phenomenon discovered by Hasselbalch and Lundsgaard is only an expression of the Schmidt-Zuntz effect as it appears in A. Schmidt's [1867] and N. Zuntz's [1867, 1868] and many other old experiments, and as is very clearly seen in Hasselbalch and Warburg's [1918] experiments and expositions. Hasselbalch and Lundsgaard showed that the reaction at constant CO_2 tension was more acid in blood than in the serum centrifuged from it. The experiments of the above mentioned authors show that the content of the serum in bicarbonate and therefore the reaction of the serum at a given CO_2 tension is a function of the CO_2 tension in the blood at the time of centrifuging, so that we cannot conclude anything about the reaction in the interior of the blood cells from a difference of reaction between serum in blood and separated serum.

The Schmidt-Zuntz phenomenon, which has been the cause of Hasselbalch and Lundsgaard's error, indicates that some kind of equilibrium prevails between the activity of the ions in blood cells and serum but it is not possible at the moment to say how this equilibrium is maintained. In the final chapter an attempt will be made to give a theoretical and experimentally workable solution of the problem on a relatively broad basis.

If the reaction of blood is determined electrically and then haemolysis produced (*e.g.* by freezing) without letting the blood come in contact with any new gas mixture, the reaction after haemolysis will show whether there is any difference of reaction between blood corpuscles and serum. The assumption involved in this reasoning—as for numerous other instances later in the chapter—is that the dissociation of the electrolytes determined by the reaction is the same at the same reaction before and after the haemolysis.

Konikoff [1913] has reported experiments in which he estimated the reaction of blood electrometrically using Hasselbalch's method. He employed a special electrode vessel with a relatively large platinum plate and assumed he avoided the oxygen error in this way (demonstration of the oxygen error was the real object of his work). Having determined the reaction of the blood he haemolysed it by freezing and found that the reaction had become much more acid.

Milroy [1917] determined the reaction in haemolysed blood at a CO_2 tension of about 40 mm. and found a $p_{H'}$ of roughly 6·60, that is about ten times as large a hydrogen ion activity as in blood serum at the same CO_2 tension.

Although Konikoff devised a special technique to avoid the O_2 error (he did not however make use of minimal immersion) and Milroy employed Höber's principle and was aware of the existence of the O_2 error, I do not hesitate to say that the results of both these investigators are misleading as they undoubtedly had considerable quantities of O_2 in their haemoglobin solutions. Neither of them attempted to prove that the oxygen was actually dissipated during the measurements and it is beyond all question that too low potentials will be obtained if the estimations are carried out with deeply immersed platinum electrodes in strongly oxygenated haemoglobin solutions.

Parsons [1917] has published some electrical determinations in haemolysed blood in which the reaction was almost the same as that usually met with in serum with a similar CO_2 tension.

L. E. Walbum [1914, p. 231] has shown that the reaction in a solution of blood corpuscles (10 blood + 90 physiological NaCl) is the same before and after haemolysis. Although the quantity of blood corpuscles in the experiments was rather small this is counterbalanced by the liquid containing them (serum + NaCl solution) being relatively poor in buffer substances. L. S. Fridericia [1920], and J. Joffe and E. P. Poulton [1920], in the papers extensively referred to in a previous chaper, calculated the reaction in blood corpuscles and serum at the same CO_2 tension in a manner which in essence is identical with that employed in the experiments about to be described, but they made the assumption that $p\lambda_{(c)}$ was the same as p_{K_I} in bicarbonate solutions of the same carbonic acid binding power, an assumption which is to a certain extent supported by Hasselbalch's [1916, 2] rather scanty estimations in dialysed haemoglobin solutions.

It is therefore hardly possible from experiments in the literature to con-

clude anything with certainty about the reaction in the blood corpuscles but I believe that Joffe and Poulton's contribution must be looked upon as the most important on this subject even though it is open to objection as $p\lambda_{(c)}$ was not experimentally determined.

If (120) $$a_{H^{\cdot}(c)} = \lambda_{(c)} \frac{100 - Q(1-D)}{100D} \times \frac{P_{CO_2}\alpha}{7{\cdot}60B}$$

is divided by (119) $$a_{H^{\cdot}(s)} = \lambda_{(s)} \frac{100 - Q(1-D)}{100} \times \frac{P_{CO_2}\alpha}{7{\cdot}60B}$$

we get $$\frac{a_{H^{\cdot}(c)}}{a_{H^{\cdot}(s)}} = \frac{\lambda_{(c)}}{D\lambda_{(s)}}, \quad \text{.....(132)}$$

which in logarithmic form becomes

$$p_{H^{\cdot}(s)} - p_{H^{\cdot}(c)} = p\lambda_{(s)} - p\lambda_{(c)} - \log D. \quad \text{.....(133)}$$

Since we have previously determined $p\lambda_{(s)}$ and D, we only require the value $p\lambda_{(c)}$ for estimating the difference in reaction between blood corpuscles and serum, which we will now attempt to determine. The determination of $p\lambda_{(c)}$ was associated with much greater difficulties than I originally expected. One of the most important was to get rid of the oxygen at the reactions and temperatures dealt with, but this was overcome to a large extent by the technique described in chapter IV. It was easy to haemolyse ox blood by repeatedly freezing so that it became completely transparent and only a trifling amount of blood corpuscles was left, but it was practically impossible by freezing alone to haemolyse horse blood so thoroughly. Even after freezing and thawing three times numerous intact blood corpuscles are present and many amorphous fragments are seen with the microscope. If the volume of the disperse phase is determined by the haematocrite—which can easily be done—it will never be found to be over 5 % of the whole system even when very concentrated blood cell suspensions are used. If a liberal amount of saponin is added to horse blood it will become completely transparent and only a few formed constituents (about 1 % in the haematocrite) can be seen with the microscope. This difficulty of haemolysing horse blood by freezing led me to work with blood haemolysed by saponin as it was found that the combined CO_2 was the same whichever of the two methods was employed as the following experiment shows.

Defibrinated horse blood was concentrated by centrifuging. Haematocrite reading 59·5.

mm. Hg CO_2	Vols. % combined CO_2	
24·3	45·2	Saponin
24·0	45·0	Freezing
79·2	72·7	,,
79·9	72·9	Saponin

In using a concentrated solution of horse blood haemolysed by saponin a new difficulty arose. When it is treated for a long time with high tensions of CO_2 it becomes very viscous and shortly afterwards a large quantity of haemoglobin crystals separate out so that the experiment has to be abandoned. It has been found that this precipitation never takes place in the first quarter

of an hour so that a preliminary treatment of the haemoglobin solution with CO_2 may be undertaken for this space of time and the experiment continued with lower CO_2 tensions (lower a_{H^+}). This crystallising out of horse haemoglobin will be reverted to in chapter XI.

In horse blood haemolysed by freezing (concentrated in the centrifuge) I have only once seen a similar crystallisation, and the haemoglobin solution was in this instance cooled to 0°.

That haemoglobin very readily crystallises out at high CO_2 tensions has been known a long time and is mentioned for example by Preyer [1871] without any particular comment. I. Setschenow [1879, p. 48] reported similar observations with strong concentrated frozen horse blood at room temperature (CO_2 and H_2SO_4 addition).

As already repeatedly mentioned the potential in an electrical determination of reaction falls when the platinum electrode has been in contact with protein solutions for some time. The drop is not large and in the course of 2–3 hours an almost constant potential seems to be reached (within $\frac{1}{2}$ millivolt), but I tried nevertheless to avoid any possible error from this cause ("deterioration" of the electrode) by developing the technique employed with L II described earlier in this work. In using this electrode vessel, in which it will be remembered the platinum electrode does not come in contact with the haemoglobin before the potentiometry is started, the potential was found to rise quickly about 10–20 millivolts in the first quarter of an hour after contact (total immersion) was established but quite irregularly. Then it became constant for a time and afterwards slowly declined. When the platinum was heated to redness before platinising the rise was much less, but as a rule a few millivolts. This must be what Parsons [1917] referred to when he wrote that it is essential to heat the electrode red hot before every determination in haemolysed blood.

In Table XXII $p\lambda_{(m)}$ is calculated from Hasselbalch's experiments with dialysed haemoglobin in weak sodium bicarbonate solution the conversion being carried out in the same way as in the preceding chapters.

$p\lambda_{(m)}$ is in agreement with $p\lambda_{(s)}$ and $p\lambda_{(c)}$ and therefore in haemolysed blood we have

$$p\lambda_{(m)} = p_{H^+} + \log \frac{P_{CO_2}\,\alpha}{7{\cdot}60} - \log \beta, \quad \text{.....(134)}$$

where β is the mean concentration of combined CO_2 (expressed in vols. % CO_2) in the haemolysed blood.

In Table XXIII[1] a number of determinations of $p\lambda_{(m)}$ in haemolysed ox blood are given. They were done with the small saturation electrode and within the same period as the experiments with blood designated series A in chapter V.

[1] The temperature corrections here and in what follows are made by adding 0·0075 to the value found for each degree over 18°, and subtracting the same amount for each degree under 18°. In the calculations from experiments in chapters V and VI 0·005 was used as the correction, but the difference is so small that I have not found it necessary to recalculate these earlier experiments with the correction employed in this chapter.

It is extremely probable that the apparent hydrogen ion exponent at this time was 0·06 too low, according to which $p\lambda_{(m)}$ in haemolysed ox blood with about 33 vols. % combined O_2 should be about 6·27.

The determinations in Table XXIV of haemolysed ox blood with 29 vols. % O_2 at 20° and 38° give respectively values of 6·26 and 6·15. They belong to series B and are carried out with the small saturation electrode.

The experiments in Table XXV were made in the large saturation electrode L I. In almost all cases a preliminary treatment with CO_2 for $\frac{1}{4}$–$\frac{1}{2}$ an hour was undertaken. Analyses of two gas mixtures are given in the table corresponding to one measurement of potential, the first relating to the last gas in the spirometer and the second to the gas in the electrode vessel..

$p\lambda_{(m)}$ at 18° referring here to horse blood cells haemolysed by saponin with about 38 vols. % combined O_2 is found to be 6·32; for ox blood haemolysed by freezing with 35 vols. % O_2 it is 6·26.

In Table XXVI measurements are given carried out with L II without heating the platinum electrode red hot before platinising. The results are quite in agreement with those reported in Table XXV, as $p\lambda_{(m)}$ in horse serum haemolysed blood cells with about 40 vols. % combined O_2 is also 6·32, while in haemolysed ox blood with about 32 vols. % it is 6·27. It is worth noticing that the final potential is not appreciably different although the platinum plate with one technique was 2–2½ hours in contact with the protein and with the other technique only $\frac{1}{4}$–$\frac{1}{2}$ hour. I should however expect that with a sufficiently extensive series of measurements a deviation of 1–2 millivolts might be demonstrated.

In Table XXVII are given measurements carried out in L II with platinum plates freshly heated to redness. The results are as will be seen a little different from those just obtained, $p\lambda_{(m)}$ in haemolysed horse blood and ox blood of similar constitution being respectively 6·35 and 6·28 at 18°. These values differ but slightly from those of the first series and the difference is hardly greater than the experimental error, a calculation of the mean error as in the case of the experiments with serum being hardly feasible.

It will be remembered that $p\lambda_{(s)}$ is 6·29 (see chapter VI) and since $p\lambda_{(m)}$ in a mixture of one part serum and three parts haemolysed blood corpuscles is 6·35 we shall not make a large error by assuming $p\lambda_{(c)} - p\lambda_{(s)}$ is 0·07, but as this difference is rather uncertain calculations have been made using (133) with values ranging from 0·05 to 0·09. The results are to be found in Table XXVIII. In the last column the values for (132) are given, the difference here being 0·07.

Table XXII. $p\lambda_{(c)}$ in dialysed haemoglobin with $NaHCO_3$ 0·025n calculated from K. A. Hasselbalch's experiment at 38°.

mm. Hg CO_2	Vols. % combined CO_2	$p_{H'}$ corrected	$p\lambda_{(m)}$
20·2	42·2	7·59	7·13
94·2	55·4	7·06	7·15
7·0	32·9	7·95	7·14

Table XXIII. $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids (freezing) determined by the small saturator electrode. Series A.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\lambda_{(m)}$ 18°	
19·5	459·9	0·5	117·3	0·6	6·51	6·19	1st day
19·0	77·7	0·5	70·5	0·7	7·09	6·21	
19·0	73·7	0·4	69·8	0·7	7·12	6·23	Ox blood
18·0	50·2	0·9	60·6	—	7·21	6·21	2nd day
18·0	50·2	0·9	63·0	0·8	7·19	6·18	Preliminary treatment with CO_2
18·0	28·6	0·5	50·2	1·8	7·36	6·18	
19·5	28·6	0·5	49·5	0·6	7·35	6·20	„ „
			Colorimetric 32				
19·0	37·4	0·5	61·1	0·7	7·33	6·20	1st day
19·5	57·1	0·5	68·6	1·4	7·23	6·23	
19·5	57·1	0·5	70·2	0·5	7·19	6·19	Preliminary treatment with CO_2
19·0	131·9	0·5	90·3	1·6	6·97	6·22	Ox blood
18·0	575·7	0·5	136·2	0·3	6·49	6·20	2nd day
19·0	82·6	0·5	79·1	0·8	7·12	6·22	
18·5	82·6	0·5	82·7	0·7	7·13	6·22	Preliminary treatment with CO_2
18·0	14·3	0·5	40·7	1·3	7·58	6·21	
					Mean	6·21	
20·0	38·6	147·0	51·9	32·2	—	—	3rd day
20·0	314·4	320·8	103·2	34·0	—	—	
19·0	103·5	0·5	77·4	1·1	7·08	6·29	1st day
19·0	40·3	0·2	—	—	7·33	—	
19·0	46·7	0·3	64·5	—	7·28	6·22	Horse blood
19·0	481·5	0·0	128·2	0·9	6·58	6·24	2nd day
			Colorimetric *circ.* 32				

Table XXIV. Washed ox blood cells haemolysed by freezing *circ.* 225 cc. + 6 cc. *n*/3 Na_2CO_3. Determinations of $p\lambda_{(m)}$ in the small saturator electrode. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H (measured)	$p\lambda_{(m)}$ 20°	$p\lambda_{(m)}$ 38°	p_H (calculated)
27. ii. 19								
38	589·2	0·9	80·8	0·4	—	—	—	6·42
20	615·8	1·0	101·5	0·7	—	—	—	6·41
38	114·3	0·3	47·2	0·6	6·90	—	6·15	6·90
20	119·5	0·3	67·2	0·5	6·95	6·26	—	6·95
38	64·7	0·5	37·3	0·2	7·05	—	6·15	7·05
20	64·6	0·5	55·4	0·7	7·15	6·27	—	7·14
28. ii. 19								
38	121·7	0·6	49·1	0·5	6·88	—	6·13	6·90
21	127·2	0·6	69·2	0·4	6·93	6·25	—	6·93
38	12·1	0·5	13·7	0·7	7·32	—	6·14	7·33
20	12·1	0·5	27·4	0·4	7·53	6·24	—	7·55
38	41·0	0·4	29·8	—	7·09	—	6·09	7·15
20	47·4	0·4	49·3	0·8	7·21	6·26	—	7·21
						Mean 6·26	6·15	
1. iii. 19								
38	36·2	140·0	22·8	28·4	—	—	—	7·10
20	37·8	146·5	38·6	29·3	—	—	—	7·20
38	112·6	565·3	42·2	29·0	—	—	—	6·87
20	117·6	590·6	62·8	—	—	—	—	6·92
38	501·3	199·1	78·4	27·2	—	—	—	6·39

Table XXV. $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids estimated with L I. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\lambda_{(m)}$ 18°		
—	46·9	0·5	—	—	—	—		
19·5	46·5	0·6	64·4	0·3	7·39	6·33	1st day	
—	106·2	0·7	—	—	—	—		
20·5	105·4	2·3	86·5	1·9	7·13	6·30	2nd day	Horse blood haemolysed with saponin
—	29·4	0·8	—	—	—	—		
19·0	29·4	0·9	50·4	0·9	7·48	6·33	3rd day	
			Colorimetric 32					
—	40·2	0·5	—	—	—	—		
20·0	40·4	1·2	64·7	1·2	7·48	6·35	1st day	Horse blood haemolysed with saponin
—	16·6	0·2	—	—	—	—		
19·0	15·3	1·2	40·7	2·1	7·65	6·31	2nd day	
—	12·3	0·5	—	—	—	—		
18·0	12·9	1·1	38·4	1·3	7·67	6·28	3rd day	
			Air from blower	37·6	Mean	6·32		
—	48·4	0·2	—	—	—	—		
19·0	47·7	0·7	66·7	?	7·40	6·34		Human blood, frozen
—	586·5	0·2	—	—	—	—		
18·0	586·5	0·4	144·9	0·2	6·57	6·27	1st day	Concentrated by centrifuging
—	60·8	0·5	—	—	—	—		
18·0	66·4	0·5	85·3	0·2	7·28	6·26		Ox blood, frozen
—	27·2	0·2	—	—	—	—		
18·0	26·7	0·7	64·1	0·2	7·55	6·26	2nd day	
			Air from blower	35·1	Mean	6·26		

Table XXVI. Determinations of $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids made with L II. The electrode was not heated to redness before each measurement. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\lambda_{(m)}$ 18°		
—	47·0	0·1	—	—	—	—		
21·0	47·0	2·9	60·7	1·4	7·35	6·31	1st day	
—	258·3	0·1	—	—	—	—		
20·0	258·8	0·2	113·0	—	6·89	6·33		Horse blood, frozen
—	74·2	0·1	—	—	—	—		
19·5	73·7	1·6	75·9	0·5	7·23	6·30	2nd day	
—	45·6	0·1	—	—	—	—		
19·0	45·2	1·2	62·1	0·5	7·40	6·33		
18·0	334·7	0·3	—	—	—	—		
—	335·2	0·3	122·1	0·1	6·78	6·31		
			Air from blower	39·7	Mean	6·32		
20·5	24·6	5·8	43·4	3·3	7·47	6·30	1st day	
—	32·2	0·3	—	—	—	—		
20·0	31·6	3·8	48·8	2·1	7·38	6·27	2nd day	Ox blood, frozen
—	101·3	0·6	—	—	—	—		
20·0	95·0	0·8	74·6	0·4	7·06	6·23	3rd day	
—	305·2	0·1	—	—	—	—		
19·0	305·2	0·7	104·4	0·3	6·73	6·28		
			Air from blower	31·8	Mean	6·27		
			Haematocrite number	72·8				

Table XXVII. Determinations of $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids made with L II. Freshly burnt out platinum electrode.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H^\cdot}$	$p\lambda_{(m)}$ 18°		
—	33·7	0·0	—	—	—	—		
19·0	33·3	1·9	53·0	1·5	7·50	6·38	1st day	
—	375·3	0·1	—	—	—	—		
19·0	375·0	0·1	117·6	0·1	6·73	6·32	3rd day	Horse blood, frozen
—	20·1	0·1	—	—	—	—		
19·0	23·6	0·6	54·5	0·0	7·63	6·33	4th day	
—	60·3	0·0	—	—	—	—		
19·0	61·6	0·0	75·1	0·0	7·36	6·37		
		Air from blower		36·3				
—	29·6	0·1	—	—	—	—		
16·0	29·7	0·1	57·5	0·0	7·50	6·31		Horse blood, frozen
		Air from blower		41·6				
—	18·9	0·3	—	—	—	—		
16·0	18·9	0·7	44·3	1·4	7·57	6·30		Horse blood, frozen
—	343·1	1·0	—	—	—	—		
18·5	342·1	2·4	120·7	1·2	6·85	6·39		Horse blood, frozen
—	89·9	0·2	—	—	—	—		
18·5	90·5	1·7	80·8	0·0	7·25	6·39		
		Air from blower		38·2	Mean	6·35		
—	388·9	0·1	—	—	—	—		
17·0	388·4	1·3	110·8	0·6	6·70	6·34		
—	22·8	0·2	—	—	—	—		
16·0	22·6	2·4	51·3	1·2	7·51	6·25		Ox blood, frozen
—	78·4	0·4	—	—	—	—		
16·0	78·9	0·2	76·3	0·1	7·17	6·28		
—	215·1	0·2	—	—	—	—		
16·0	214·5	0·6	99·8	0·1	6·82	6·25		
		Air from blower		27·8	Mean	6·28		

Table XXVIII. The difference of reaction between blood cells and serum (horse blood at room temperature).

$p\lambda_{(s)} - p\lambda_{(c)}$		−0·05	−0·06	−0·07	−0·08	−0·09	−0·07
		$p_{H^\cdot(s)} - p_{H^\cdot(c)}$					$\frac{a_{H^\cdot(c)}}{a_{H^\cdot(s)}}$
$p_{H^\cdot(s)}$	$-\log D$						
6·50	*+0·055*	+0·005	−0·005	−0·015	−0·025	−0·035	0·966
6·88	*+0·097*	+0·047	+0·037	+0·027	+0·017	+0·007	1·06
7·27	*+0·155*	+0·105	+0·095	+0·085	+0·075	+0·065	1·22
7·40	*+0·180*	+0·130	+0·120	+0·110	+0·100	+0·090	1·29
7·60	*+0·222*	+0·172	+0·162	+0·152	+0·142	+0·130	1·42
7·90	*+0·301*	+0·251	+0·241	+0·231	+0·221	+0·211	1·70

A dotted line is drawn through the table which indicates the reactions at which blood cells and serum have the same reaction. It will be seen this is the case between 6·50 and 6·88. Fig. 11 is a graphic representation of the table, the apparent hydrogen ion exponents of serum being the abscissae and the differences between the exponents of serum and blood cells the ordinates. For clearness only the curves relating to $p\lambda_{(c)} - p\lambda_{(s)}$ 0·05, 0·07 and 0·09 are given.

It will be further seen from the tables and curves that the apparent hydrogen ion activity is larger in blood corpuscles than in serum at serum reactions more alkaline than $p_{H^{\cdot}} = 6{\cdot}9$ ($a_{H^{\cdot}} = 1{\cdot}26 \times 10^{-7}$) and that the difference increases with the hydrogen ion exponent.

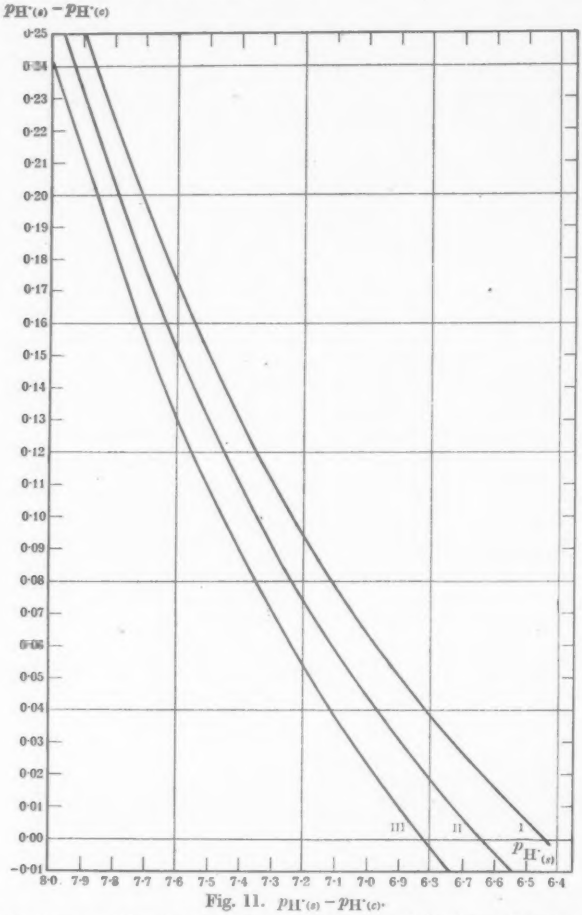


Fig. 11. $p_{H^{\cdot}(s)} - p_{H^{\cdot}(c)}$.

As D in human blood at 38° is the same as D in horse blood at 18° the above considerations will also apply to this species of blood if $p\lambda_{(c)} - p\lambda_{(s)}$ is the same. In the tables there is only one measurement of human blood at room temperature to be found but this fits in quite well in the horse blood series. The question can however only finally be settled by many estimations.

According to Fr. Kraus' [1898] experiments D for ox blood appears to be 1·00 at $p_{H^{\cdot}}$ about 6·8. When this is compared with the determinations of $p\lambda_{(m)}$ in ox blood the same reaction should exist in serum and blood cells at this reaction because $p\lambda_{(c)}$ and $p\lambda_{(s)}$ are very nearly identical in such blood.

It appears also from the few determinations of D for ox blood which are reported in a preceding chapter that the difference of reaction between blood cells and serum increases with the $p_{H'}$.

If we assume that the dissociation of electrolytes which varies with the reaction is the same at a similar reaction before and after haemolysis we can draw conclusions from the combination of CO_2 at the same CO_2 tension, before and after haemolysis, about a possible difference of reaction between blood cells and serum. The combination of CO_2 increases with the apparent hydrogen ion activity in serum and in the fluids of the blood cells but it increases most in the latter case. If the same $p_{H'}$ persists after haemolysis as there was in serum and blood cells before haemolysis (*e.g.* in horse blood at $p_{H'}$ 7·60) then the CO_2 combination is not altered. If however there was a higher $a_{H'}$ in blood cells than in serum before haemolysis, $a_{H'}$ will be midway between the original reaction of the serum and blood cells under the given conditions after haemolysis and less CO_2 will be combined with the electrolytes varying with the reaction in blood cells but more with those in serum. The result will be that, altogether, less CO_2 will be combined after haemolysis than before if the volumes of blood cells and serum are equal.

I have only made a few experiments (about ten) with ox blood with this object in view but they all go to show that less CO_2 is combined after haemolysis than before, at alkaline reactions. At reactions about 6·30 the combined CO_2 was almost or actually the same in blood before and after haemolysis. In some experiments in which the osmolar concentration of the blood was a little diminished the difference was smaller than in ordinary blood. In an isolated experiment where the osmolar concentration of the blood was rather increased the opposite was the case. These effects of the changes in the osmolar concentration are in accordance with the theory, as D varies with the variations in volume of the blood corpuscles.

As will be noticed there is a disagreement, although a small one, between the results attained by the first and last mentioned principles for the determination of the difference of reaction in ox blood. From the first principle we concluded that the reaction was identical in serum and corpuscles at $p_{H'}$ 6·8 while the last pointed to the fact that there was no difference as far as $p_{H'}$ 6·3. Further experiments are needed to clear up the matter.

In Table XXIX some of the experiments[1] mentioned are given. Haemo-

[1] The experiment at 38° was particularly interesting as the relation between oxy-haemoglobin and reduced haemoglobin is not altered by haemolysis. This result was supported by several experiments with saponin haemolysed blood which I hope later to have the opportunity of publishing. The phenomenon itself is not without interest because it indicates that the quantity and kind of salt does not play so great a part in determining the form of the O_2 combination curve of haemoglobin as Barcroft [1914] and his collaborators imagined. That the dilution of the haemoglobin due to the haemolysis plays no great part in the relation between oxy-haemoglobin and reduced haemoglobin was only to be expected because they are both diluted to the same degree and there should thus be no change in the extent of oxygenation of the haemoglobin either according to the interpretation of the process expressed by G. Hüffner [1901] in his later papers or by A. V. Hill [1910, 1913, 1921].

If we could estimate the degree of oxygenation with sufficient accuracy we might however expect to find a little greater oxygenation after haemolysis than before at reactions more alkaline than $p_{H'}$ 6·8 because the extent of oxygenation is a function of the reaction; cf. Chr. Bohr, K. A. Hasselbalch and A. Krogh [1904], R. A. Peters [1914, 2], K. A. Hasselbalch [1916, 2] and L. J. Henderson [1920].

Table XXIX.

mm. Hg		Vols. % combined CO_2		Vols. % combined O_2		$pH^{(c)}$
CO_2	O_2	in blood	in haemoglobin	in blood	in haemoglobin	calculated
		Blood and water-haemolysed blood at 19°				
14·9	151·6	24·1	—	15·0	—	7·25
14·9	151·6	—	21·6	—	14·6	—
53·0	143·3	—	35·8	—	14·4	—
53·0	143·3	37·5	—	—	—	6·98
144·3	163·8	—	47·3	—	14·4	—
144·3	163·8	48·6	—	14·5	—	6·73
164·5	313·2	50·5	—	14·6	—	6·29
164·5	313·2	—	48·5	—	14·7	—
467·6	256·2	—	60·5	—	14·5	—
467·6	256·2	61·6	—	14·3	—	6·32
731·5	trace	—	64·9	—	—	—
731·5	„	67·0	—	1·3	1·4	6·15
		Blood and water-haemolysed blood at 19°				
10·7	153·0	25·9	—	8·0	—	7·60
10·7	153·0	—	24·1	—	8·1	—
34·1	148·1	35·4	—	8·0	—	7·22
34·1	148·1	—	32·9	—	7·9	—
67·3	141·3	40·0	—	—	—	6·99
67·3	141·3	—	38·7	—	8·2	—
135·3	127·0	46·1	—	7·7	—	6·75
135·3	127·0	—	44·8	—	7·4	—
133·0	126·4	45·8	—	—	—	6·76
133·0	126·4	—	44·6	—	—	—
405·9	69·9	54·3	—	—	—	6·33
405·9	69·9	—	54·3	—	—	—
736·0	trace	59·4	—	3·3	—	6·13
736·0	„	—	60·0	—	1·6	—
		Blood and blood haemolysed by freezing at 18°				
17·4	152·1	47·1	—	16·0	—	7·65
17·4	152·1	—	45·1	—	15·6	—
98·4	135·1	—	72·8	—	—	—
96·1	135·6	72·1	—	15·8	—	7·07
430·4	64·8	—	96·5	—	12·9	—
429·2	65·3	96·3	—	14·0	—	6·50
		Blood and water-haemolysed blood at 18°				
37·2	150·4	—	28·0	—	12·9	—
37·2	150·4	30·1	—	12·9	—	7·11
37·2	150·4	—	28·7	—	13·0	—
37·2	150·4	30·2	—	12·8	—	7·11[1]
		[1] CO_2 preliminary treatment for half an hour.				
		Blood and water-haemolysed blood at 38°				
15·3	144·5	—	12·9	—	8·1	—
15·3	144·5	13·4	—	7·8	—	7·25
42·3	138·6	—	19·5	—	7·7	—
42·3	138·6	20·2	—	7·4	—	6·98
114·6	124·2	—	27·0	—	7·1	—
114·6	124·2	27·5	—	7·4	—	6·62
213·7	100·1	32·6	—	6·2	—	6·46
213·7	100·1	—	32·0	—	6·2	—
455·6	48·0	40·1	—	2·3	—	6·23
455·6	48·0	—	41·3	—	2·2	—

lysis was brought about either by freezing or by the addition of water. When haemolysis by water was completed sufficient NaCl was added to make the salt content up to 0·9 % again.

I. Setschenow [1879, p. 44] reported the following experiment at 37°–37°·5. Emulsion of dog blood corpuscles:

514·6 mm. CO_2, total CO_2 in 50·18 cc. = 50·31 cc. (0°, 1 mtr.).

After freezing:

513·3 mm. CO_2, total CO_2 in 50·18 cc. = 49·98 cc. (0°, 1 mtr.).

From this $p_{H'}$ is 6·22 if $\Psi = 15$ and $p\lambda_{(m)} = 6{\cdot}20$, at which reaction there seems to be the same reaction in dog blood cells and serum.

RÉSUMÉ.

The apparent hydrogen ion activity in horse blood corpuscles has been determined.

CHAPTER VIII

THE DETERMINATION OF THE FIRST DISSOCIATION CONSTANT OF CARBONIC ACID AND THE DEVIATION COEFFICIENTS OF THE BICARBONATE ION.

In the foregoing chapters we have determined the value of $p\Lambda_{(s)}$ and $p\Lambda_{(c)}$, and it will now be interesting to inquire into the factors which control these constants rather more closely.

It will be remembered that in chapter III it was shown that the apparent activity coefficient of the continuous phase of serum and of the blood cell fluid participates in the constants and a rather large number of determinations have therefore been performed of the apparent activity coefficients in salt solutions. I have at several points pursued the investigations further than was absolutely necessary for the problem being dealt with, because it may be of particular interest from a purely physico-chemical standpoint, especially since the appearance of Bjerrum's theory.

The first dissociation constant of carbonic acid has been determined by the conductivity method by J. Walker and W. Cormack [1900] and by J. Kendall [1916] on the basis of experiments carried out by himself, by Pfeiffer[1], by Knox[1] and by Walker and Cormack. The determinations of Walker and Cormack and Kendall are better than the earlier ones (Pfeiffer's and Knox's) and therefore the values calculated from them are the most valuable. The molecular conductivity of the bicarbonate ion at "infinite dilution" comes into the calculation. This value is obtained by extrapolation from conductivity determinations of sodium bicarbonate solutions (and also calcium bicarbonate solutions), but it seems to the author that Walker and Cormack and Kendall have not executed this extrapolation in a satisfactory

[1] Cited from Kendall [1916].

manner. A slight error in the extrapolation is of hardly any consequence in the determination of the first dissociation constant of carbonic acid because the molecular conductivity of the bicarbonate ion has to be added to the much greater molecular conductivity of the hydrogen ion and it is the sum of these quantities which is used as a factor in the calculation (see chapter I). But if it is required to determine the conductivity coefficient an error in μ_∞ will have a great effect particularly in weak solutions. The question as to how μ_∞ should be obtained by extrapolation from conductivity determinations of salt solutions has up to the present been the subject of much controversy but I am unacquainted with any well-grounded theoretical method of performing the extrapolation. The best way must therefore be to obtain a relation between a function of the salt concentration and a function of the molecular conductivity which is the equation for a straight line. We can then determine μ_∞ either graphically or by the method of least squares.

It will as a rule be much the most convenient method to perform the extrapolation graphically but the method of least squares has the advantage of giving an estimation of the accuracy of the determinations.

That I only occasionally estimate the error in this chapter is because the value obtained is only of real significance if a systematic error can be excluded and a determination of the error can only be used in comparing results obtained by the same experimentalist with the same technique in a similar process. In the majority of cases the graphic method carried out in a suitable way, coupled with a good idea of the accuracy of the technique, will give more valuable information than the more arduous determination of the error.

Kohlrausch (cited from Lehfeldt [1908, p. 61]) recommends that the cube roots of the concentrations be plotted as abscissae and the molecular conductivities as ordinates. The curve will then be a straight line for many salt solutions. This kind of extrapolation has proved very suitable in the case of Walker and Cormack's determinations but particularly so for Kendall's measurements of conductivity in sodium bicarbonate solutions.

In Table XXX Walker and Cormack's and also Kendall's results are given. In the first column the number of litres in which a gram equivalent is present are recorded; in the second column, the concentration in terms of normality; in the third the cube root of this amount; in the fourth, the equivalent conductivity. In Fig. 12 these values are plotted, $\sqrt[3]{c}$ as abscissae and μ_c expressed in reciprocal ohms as ordinates. The ordinates which refer to sodium bicarbonate are on the left, the numbers referring to the calcium bicarbonate series are on the right. It will be seen from the figure that the curve which represents Kendall's measurements of sodium bicarbonate solutions corresponds extremely well to a straight line. Extrapolation can therefore be undertaken with very considerable certainty and it gives $\mu_\infty = 99{\cdot}2$ reciprocal ohms (25°), while Kendall himself made the extrapolation to be 97·5. From this—using the same corrections as Kendall did—μ_∞ at 18° is 86·3 reciprocal ohms, while Walker and Cormack's determinations on extra-

polation give 88·1 reciprocal ohms. The figure shows that the extrapolation of Walker and Cormack's measurements is rather uncertain, the two estimations in the most dilute solutions being a little high. The want of agreement between Walker and Cormack's and Kendall's results is presumably due to this cause and I shall regard Kendall's as correct.

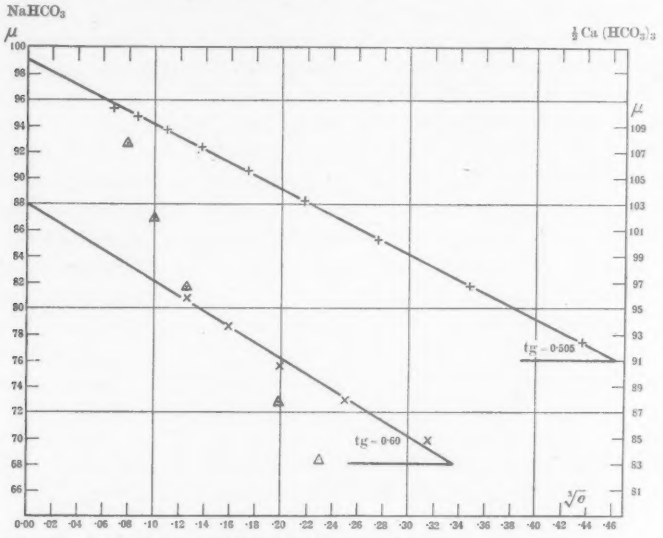


Fig. 12. Conductivity of sodium bicarbonate solutions.
Upper curve from Kendall's measurements; lower curve from Walker and Cormack's measurements.
△ ½ calcium bicarbonate.

The curve of the calcium bicarbonate experiments (triangles) demonstrates that only the three experiments with the strongest solutions lie on a straight line—the experiments with the two weakest solutions lie considerably above. Extrapolation, having regard to these two points, gives quite an unreasonable value for μ_∞, and I therefore think it cannot be performed with these experiments.

If we calculate the molecular conductivity of carbonic acid from Kendall's experiments with sodium bicarbonate (carbonic acid being regarded as monobasic) we get

at 25° 395·5,
,, 18° 355·8,
,, 0° 265·6.

Using these figures, values for the first dissociation constant of carbonic acid are obtained which diverge in the third decimal place from those calculated by Kendall [1916]. In Table XXXI Walker and Cormack's [1900] and Kendall's results are recalculated. Pfeiffer's and Knox's experiments are not given and the reader is referred to Kendall's paper for these. The small

difference between Kendall's value for μ_∞ for carbonic acid and mine is without real significance in the calculations from these experiments.

The mean of Walker and Cormack's and Kendall's determinations at 18° of the first dissociation constant of carbonic acid is $3{\cdot}1 \times 10^{-7}$, from which $p_{K_1} = 6{\cdot}509$. At 25° we obtain from Kendall's experiments $K_1 = 3{\cdot}47 \times 10^{-7}$, $p_{K_1} = 6{\cdot}460$, and at 0°, $K_1 = 2{\cdot}21 \times 10^{-7}$, $p_{K_1} = 6{\cdot}656$; p_{K_1} therefore decreases 0·20 between 0° and 25° which is equivalent to 0·008 per degree.

By thermodynamic methods it is possible to determine the change in p_{K_1} with temperature when the heat of ionisation of carbonic acid is known. Julius Thomsen has estimated it at 2800 calories, from which the change in p_{K_1} can be calculated by van 't Hoff's equation (cf. Henderson [1909], Hasselbalch [1916, 2] and Kendall [1916]), and turns out to be 0·0065 per degree, which is in good agreement with Kendall's direct determination. Kendall has pointed out that the heat of hydration of CO_2 participates in Thomsen's determinations and that the calculation of the change of the dissociation exponent with temperature according to van 't Hoff's equation is only correct if the degree of hydration is independent of the temperature. He thinks however he is justified in concluding, from the good agreement between the calculated and experimental values, that the calculation is permissible—that is to say that the change in the degree of hydration is negligible in this connection.

From Fig. 12 it appears that the relation between the molecular conductivity of sodium bicarbonate and the concentration of the salt is expressed by the following equation:

$$-50{\cdot}5\sqrt[3]{c} + 99{\cdot}2 = \mu_v, \quad \text{.....(135)}$$

from which we get $$f_\mu = \frac{\mu_v}{99{\cdot}8} = 1 - 0{\cdot}509\sqrt[3]{c}. \quad \text{.....(136)}$$

No general relation is known between the conductivity coefficient of a salt and its activity coefficient but Hasselbalch, in his frequently cited paper [1916, 2], has carried out determinations which permit of the estimation of the apparent activity coefficient of the bicarbonate ion.

The experiments referred to were electrical estimations of hydrogen ion activity in sodium bicarbonate solutions of known concentration with simultaneous estimation of the CO_2 tension with which the solutions were in equilibrium. From these experiments Hasselbalch determined p_{K_I} (Hasselbalch) with the help of the equations developed in chapter III. In Table XXXII these experiments will be found recalculated to give our $p_{K'_1}$ and, as before, the values corrected for depressed hydrogen tension as well as the uncorrected are given. Hasselbalch's experiments were performed by his own method with a plate electrode. For the sake of clearness we will postpone the further examination of these experiments.

Hasselbalch's experiments were carried out, as repeatedly stated, on the principle he evolved himself. I began therefore by repeating these experiments with the earlier described technique on Höber's principle, widening

the scope of the investigation by including sodium bicarbonate solutions which in addition contained NaCl or KCl in the concentrations 0·0718*n*, 0·145*n*, and 0·291*n*. These experiments are given in Tables XXXIII–XXXVIII and belong to series A but were done early in this series. I do not think they are subject to any systematic error but I cannot express myself with absolute certainty on this point.

In the first column of the tables the constitution of the solutions is given. The bicarbonate content of the solutions was estimated with the exhaust pump, at least two samples being used and usually several more. The error of this method is negligible compared with that of the electrical method. NaCl and KCl were added to the solutions in measured quantities, the salts being washed from a watch-glass into a measuring flask containing the bicarbonate solution. In a number of cases NaOH was used instead of $NaHCO_3$ and the solutions were therefore treated with CO_2 for a quarter of an hour before the actual saturation. It was impossible to demonstrate any difference between solutions prepared with the bicarbonate and the hydroxide.

In calculating the combined CO_2 (B), dissociation of the bicarbonate into the monocarbonate is taken into account.

In accordance with what was said in chapter II we have

$$S_1 = \frac{a_{H\cdot}}{K'_2 + a_{H\cdot}} B,$$

where S_1 is the amount of combined CO_2 corresponding to the bicarbonate expressed in vols. %, and B is the total amount of combined CO_2 expressed in the same way.

Since S_2 is the amount of combined CO_2 corresponding to the monocarbonate, and T is the amount of CO_2 in vols. % which would be combined if the combined CO_2 was in the form of bicarbonate, we have

$$S_1 + 2\,S_2 = T, \quad \text{.....(137)}$$

from which with the aid of (102) we get

$$B = \frac{\frac{a_{H\cdot}}{K'_2} + 1}{\frac{a_{H\cdot}}{K'_2} + 2}\, T. \quad \text{.....(138)}$$

When B and $a_{H\cdot}$ are known, therefore, T can be calculated and inversely B can be calculated from T and $a_{H\cdot}$. In the pumping out experiments for the determination of $C_{HCO'_3}$, B was put equal to T when $p_{H\cdot}$ was below 7·70; if it was above, (138) was used in the calculation, K'_2 being taken as 10^{-10}. In calculating $p_{K'_1}$, T was put equal to B when $p_{H\cdot}$ was less than 7·90; when greater, (138) was used. In these last experiments equation (103) was also used instead of (95) (in logarithmic form) for calculating $p_{K'_1}$.

In Table XXXIX the results of this series of measurements are compared. They show that $p_{K'_1}$ undoubtedly decreases with increasing salt concentration and that the effect of the sodium ion on the constant is more pronounced than that of the potassium ion. The measurements in pure sodium bicarbonate

solutions in the case of the two weakest concentrations ($T = 8{\cdot}88$ and $T = 17{\cdot}96$) are uncertain because the conductivity of these solutions was so slight that the resistance in the exit tap was very great and the oscillations of the capillary electrometer were too small.

In Table XXXVIII there are a number of determinations of $p_{K'_1}$ at room temperature in sodium bicarbonate solutions with the addition of equivalent amounts of NaCl or KCl. The experiments with the two series of salt solutions were performed on the same day and with the same electrode and they are therefore as comparable as possible, with the technique employed.

In Tables XL and XLIV experiments belonging to series B are given. $p_{K'_1}$ is here determined for sodium bicarbonate solutions partly with the addition of salt, at room temperature and at 38°.

In Table XLI measurements are given, carried out with the Hasselbalch electrodes E III and E VI, employed as Höber electrodes in the manner described in chapter IV, at room temperature. I consider these last measurements to be the best of all from a technical point of view, and I shall examine them a little more in detail. In the first place it will be observed that $p_{K'_1}$ for pure sodium bicarbonate solutions decreases with the concentration.

$n/100$ $NaHCO_3$ gives $p_{K'_1}$ 6·400
$n/40$ „ „ „ 6·393
$n/20$ „ „ „ 6·335
$n/10$ „ „ „ 6·279

In potassium bicarbonate solutions for

$n/100$ $KHCO_3$, $p_{K'_1} = 6{\cdot}413$
$n/10$ „ „ $= 6{\cdot}317$

If we compare the solutions with salt concentrations of $n/10$ we get

$n/10$ $NaHCO_3$, $p_{K'_1} = 6{\cdot}279$
$n/10$ $KHCO_3$, $p_{K'_1} = 6{\cdot}317$
$n/20$ NaCl + $n/20$ $NaHCO_3$, $p_{K'_1} = 6{\cdot}297$
$n/20$ KCl + $n/20$ $KHCO_3$, $p_{K'_1} = 6{\cdot}312$
$n/10$ NaCl + $n/1000$ $NaHCO_3$, $p_{K'_1} = 6{\cdot}313$

It is seen therefore that $p_{K'_1}$ for $n/10$ sodium salt solutions is rather less than the constant for $n/10$ potassium salt solutions. This is still more noticeable in the determinations in $n/5$ salt solutions, and from the latter it also appears that $p_{K'_1}$ for mixtures of Na and K salts lies between those for the pure salts.

$n/10$ NaCl + $n/10$ $NaHCO_3$ gives $p_{K'_1} = 6{\cdot}246$
$n/10$ KCl + $n/10$ $KHCO_3$ „ „ $= 6{\cdot}260$
$n/10$ KCl + $n/10$ $NaHCO_3$ „ „ $= 6{\cdot}282$

Turning again to the $n/10$ solutions it will be noticed that $p_{K'_1}$ seems to be a little greater for solutions containing NaCl than for those containing equivalent amounts of $NaHCO_3$. The difference in $p_{K'_1}$ for solutions containing

pure $NaHCO_3$ and for those containing equal parts of NaCl and $NaHCO_3$ is however so small that it certainly falls within the experimental error, but the difference between $n/10$ $NaHCO_3$ and $n/10$ NaCl + $n/1000$ $NaHCO_3$ appears to be real. I think that in this case the method of least squares ought to be used.

From equation (89) in logarithmic form we get

$$p_{K'_1} = p_{K_1} + \log F_a(HCO'_3).$$

According to Bjerrum (cf. equation (58))

$$\log f_a(HCO'_3) = -k\sqrt[3]{c}.$$

If we put $$F_a(HCO'_3) = f_a(HCO'_3),$$

which is permissible at $n/10$ concentration, we get

$$p_{K'_1} = p_{K_1} - k\sqrt[3]{c}, \quad \text{.....(139)}$$

where c is the molar concentration of the cations and k is the constant in Bjerrum's above cited equation, while p_{K_1} is the negative logarithm of the first dissociation constant of carbonic acid.

If we have a series of equations of the form

$$ax + y = l, \quad \text{.....(140)}$$

we can calculate the constants which fulfil the conditions best with the following equations:

$$x = \frac{n\Sigma al - \Sigma a\Sigma l}{n\Sigma a^2 - (\Sigma a)^2}, \quad \text{.....(141)}$$

$$y = \frac{\Sigma l\Sigma a^2 - \Sigma al\Sigma a}{n\Sigma a^2 - (\Sigma a)^2}. \quad \text{.....(142)}$$

If we employ the above equations in connection with all the 26 experiments which only contain $NaHCO_3$ in the series recorded we obtain

$$p_{K_1} = 6{\cdot}512,$$
$$k = 0{\cdot}475.$$

An estimate of the mean error of $p_{K'_1}$ can be obtained by putting the values found for it and k in (139), and determining the deviations from the above value, thus

$$M = \sqrt{\frac{7519}{25}} \times \frac{1}{100} = 0{\cdot}0173.$$

For a series of nine experiments we may therefore expect a mean error of 0·0058. Thus for such a series of measurements with $n/10$ $NaHCO_3$

$$p_{K'_1} = 6{\cdot}291 \pm 0{\cdot}0058.$$

In the experiments with the solution of $n/10$ NaCl + $n/1000$ $NaHCO_3$

$$p_{K'_1} = 6{\cdot}313 \pm 0{\cdot}0045.$$

The difference is therefore $0{\cdot}022 \pm 0{\cdot}0073$ and in all probability it is a real one.

It is an obvious consequence of Bjerrum's activity theory, as it has been expounded in chapter I and the present chapter, that the activity coefficient in a solution of a binary salt consisting of two monovalent radicles shall not vary[1] if the solution is diluted with another which contains an equivalent amount of a similar salt having no ions in common with the first.

[1] Apart from secondary effects represented by the difference in k in equation (46).

According to a view widely held by physiologists, which for example is put forward by Hamburger [1902], Hedin [1915] and Ege [1920], the dissociation of a monovalent salt will increase if the salt solution is diluted with another monovalent salt having no ions in common with the first, even if the two solutions are equivalent. This view depends however on an incorrect application of Arrhenius' [1888] theory of "isohydric" solutions, but I was myself involved in it until Prof. Bjerrum kindly pointed out the fallacy to me. In view of the general acceptance it has received I will enter a little more deeply into the question regarding for the time being Arrhenius' classical theory of the incomplete dissociation of salts as correct.

Let us consider a $n/1$ solution of NaCl and a $n/1$ solution of KI.

Then according to Arrhenius' theory extended to isohydric dissociation

$$\frac{C_{Na^{\cdot}} + C_{Cl'}}{C_{NaCl}} = k,$$

and

$$\frac{C_{K^{\cdot}} + C_{I'}}{C_{KI}} = k.$$

The equilibria in a $n/1$ KCl solution and a $n/1$ NaI solution will be given by

$$\frac{n/1\,\gamma K^{\cdot}\; n/1\,\gamma Cl'}{n/1\,KCl\,(1-\gamma)} = k,$$

and

$$\frac{n/1\,\gamma Na^{\cdot}\; n/1\,\gamma I'}{n/1\,NaI\,(1-\gamma)} = k,$$

γ being the (common) degree of dissociation of the salts.

If we mix some NaCl solution with some KI solution, *e.g.* equal parts, an equilibrium will be established which will satisfy all the four equations. This is only possible at a quite definite degree of dissociation of the different salts as the determining equations can only have one solution.

Assuming that the dissociation does not change on mixing the solutions, we have

$$C_{Na^{\cdot}} = C_{Cl'} = C_{K^{\cdot}} = C_{I'} = n/2\gamma,$$

and

$$C_{NaCl} = C_{KI} = n/2\,(1-\gamma).$$

Now NaCl and KI will react with each other and equal quantities of NaI and KCl will be formed if the same laws hold for all the undissociated salts in question. Thus we have the following equilibria:

$$C_{NaCl} = C_{KI} = C_{NaI} = C_{KCl} = n/4\,(1-\gamma),$$

$$\frac{n/2\,\gamma Na^{\cdot}\; n/2\,\gamma Cl'}{n/4\,NaI\,(1-\gamma)} = k,$$

$$\frac{n/2\,\gamma K^{\cdot}\; n/2\,\gamma I'}{n/4\,KI\,(1-\gamma)} = k,$$

$$\frac{n/2\,\gamma Na^{\cdot}\; n/2\,\gamma I'}{n/4\,NaI\,(1-\gamma)} = k,$$

$$\frac{n/2\,\gamma K^{\cdot}\; n/2\,\gamma Cl'}{n/4\,KCl\,(1-\gamma)} = k,$$

which are correct in accordance with the four original equations. As only one state of equilibrium is possible the above mathematical reasoning is proof that no change in the dissociation of the salts takes place on mixing.

Finally it may be stated that as far as the proof is concerned it is immaterial whether the dissociation equations are really equal to a constant or only to a function of the total salt concentration, provided that all the equations are equal to the same quantity.

Equation (139) is a straight line and corresponds to one in a rectangular coordinate system in which the abscissae are $\sqrt[3]{c}$ and the ordinates $p_{K'_1}$, the straight line cutting the ordinate axis at p_{K_1}, while the tangent of the angle made by the straight line with the abscissa axis measured in the second or fourth quadrant is equal to x. In Fig. 13 $\sqrt[3]{c}$ is plotted as abscissae and $p_{K'_1}$ as ordinates, the total cation concentration in the solutions which contain either sodium salts or potassium salts alone being calculated. Only the experiments for which $\sqrt[3]{c}$ is given in the tables are plotted in the figure.

Hasselbalch's experiments (the two lowest dotted curves) are seen to be about 0·09 lower in the coordinate system than mine, so that Hasselbalch's p_{K_1} (18°) is 6·412, p_{K_1} (38°) is 6·302, p_{K_1} (18°) $-$ p_{K_1} (38°) $=$ 0·11, while k is 0·52.

The continuous line refers to the series of pure sodium bicarbonate solutions fully discussed above, while the uppermost dotted line is the one that best represents, as far as one can judge, all the experiments with sodium salts (the experiments with $n/1000$ $NaHCO_3$ are however omitted). This line gives

$$p_{K_1} = 6{\cdot}514,$$
$$k = 0{\cdot}46.$$

There is only slight uncertainty in drawing the line and without doubt only a small real error. It is worth noting that the equation

$$-\log F_a(HCO'_3) = 0{\cdot}46\sqrt[3]{c} \qquad \text{.....(143)}$$

appears to hold up to $0{\cdot}4n$ with good approximation.

The lowest dotted curve is parallel with the uppermost but 0·11 lower. It represents the determinations at 38° tolerably well. It will be observed that the change in the constant per degree is 0·0055 in Hasselbalch's experiments and also in mine, therefore considerably less than the change found by Kendall [1916], namely 0·008, and rather less than that obtained by thermodynamic methods with Thomsen's value for the heat of reaction, namely 0·0065. The uppermost dotted curve represents the determinations of $p_{K'_1}$ for pure potassium salt solutions. It gives $p_{K_1} = 6{\cdot}497$ and $k = 0{\cdot}38$. The value found for p_{K_1} is presumably a little too low because it is hardly possible there can be any real difference between p_{K_1} in sodium and potassium solutions, but there are too few determinations in low concentrations in the series to enable us to attribute the same importance to the constants for potassium as to those for sodium salts.

From their experiments on the reaction of $CaCl_2$ solutions saturated with $Ca(HCO_3)_2$ Bjerrum and Gjaldbaek [1919] calculated the "reaction constant" of calcium carbonate as

$$\log K = -5{\cdot}02 \text{ at } 18°.$$

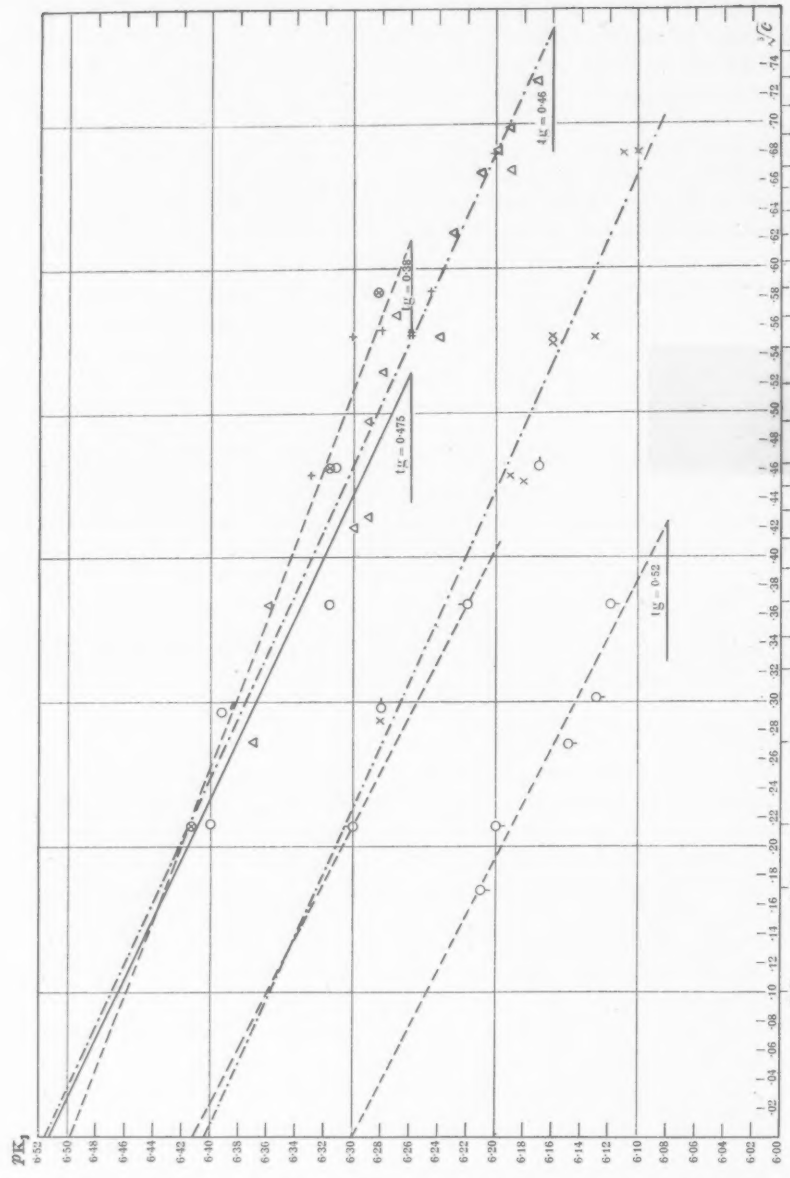


Fig. 13.

This experimental determination they compared with a calculation of the reaction constant by means of the following equation, Bjerrum and Gjaldbaek's equation (28),

$$\log K = \log K_1 + \log Kp - \tfrac{1}{2} \log K', \quad \text{.....(144)}$$

where K_1 is the first dissociation constant of carbonic acid ($\log K_1$ is therefore equal to $- p_{K_1}$), Kp is the "solubility" constant of CO_2 which is estimated as

$$\log Kp = - 1{\cdot}38,$$

and K' is a constant, which we shall shortly consider a little more closely, the value used by Bjerrum and Gjaldbaek being

$$\log K' = - 5{\cdot}68.$$

Bjerrum and Gjaldbaek employed the mean of Walker and Cormack's and Kendall's determinations, namely $- 6{\cdot}51$, as the value for $\log K_1$, from which they obtained

$$\log K = - 5{\cdot}05,$$

a figure which therefore was only 0·03 less than that determined directly.

Now it will be seen that equation (144) reversed can be used for the calculation of $\log K_1$ if $\log K'$ is known, and that by substituting the value found, namely $\log K' = - 5{\cdot}02$, we obtain

$$\log K_1 = - 6{\cdot}48.$$

This value agrees extremely well with that previously obtained from Walker and Cormack's and Kendall's experiments, and with those I myself determined, but it will be shown the agreement can be made still better.

The constant K' determines the following equilibrium, Bjerrum and Gjaldbaek's equation (15),

$$\frac{a_{Ca^{\cdot\cdot}} \cdot a^2_{HCO_3}}{P_{CO_2}} = K'^{1}. \quad \text{.....(145)}$$

Apropos of this they write:

"The ionic activity (a) can be calculated by multiplying the ionic concentration (c) by the ionic activity coefficient (F_a). For the monovalent bicarbonate ion the activity coefficient is approximately given by

$$\log F_a = - 0{\cdot}3 \sqrt[3]{c_{Ion}},$$

and for the divalent calcium cation it is given by

$$\log F_a = - 2 \times 0{\cdot}3 \sqrt[3]{c_{Ion}},$$

c_{Ion} standing for the ionic normality of the solution. (145) now assumes the following form:

$$\frac{C_{Ca^{\cdot\cdot}} \cdot C^2_{HCO'_3}}{P_{CO_2}} = K' \cdot 10^{1{\cdot}2 \sqrt[3]{c_{Ion}}}. \quad \text{.....(146)}$$

If we call the equivalent concentration of the calcium carbonate which Schlösing[2] found dissolved C, we have

$$C_{Ca^{\cdot\cdot}} = \tfrac{1}{2}C, \quad C_{HCO'_3} = C, \quad C_{Ion} = C.$$

[1] Bjerrum and Gjaldbaek substitute p_{CO_2} for my P_{CO_2}, and naturally their numbering of equations is different from that given here.

[2] Cited from Bjerrum and Gjaldbaek [1919].

By substitution and taking logarithms (146) becomes

$$3 \log C - \log P_{CO_2} - 1{\cdot}2 \sqrt[3]{c} - \log 2 = \log K'. \quad \text{.....(147)"}$$

Bjerrum and Gjaldbaek calculate the values of log K' with the help of (147) from Schlösing's[1] experiments on the solubility of calcium carbonate at different CO_2 tensions, and their results are recorded in Table XLV in the first three columns. It will be noted they have calculated the activity of the bicarbonate ion from the equation

$$\log F_a (HCO'_3) = - 0{\cdot}30 \sqrt[3]{C}, \quad \text{.....(148)}$$

but as we have previously found the value 0·3 is too low their tables have been recalculated with the equation

$$3 \log C - \log P_{CO_2} - 1{\cdot}52 \sqrt[3]{c} - \log 2 = \log K', \quad \text{.....(149)}$$

having assumed that the constant in the equation for calculating the activity coefficient of the bicarbonate ion in calcium carbonate solutions is the same as in sodium salt solutions (see above). The values obtained have been put in the fourth column of Table XLV. Log K' becomes $- 5{\cdot}709$ and it will be observed that the agreement between the individual experiments is better calculated in this way than by Bjerrum and Gjaldbaek's method. Schlösing's[1] experiments were carried out at 16° and Bjerrum and Gjaldbaek therefore convert the values to 18° using a temperature coefficient derived from experiments of R. C. Wells[1]. Correcting the constant in the same way we obtain $\log K' = - 5{\cdot}749$ at 18°.

Now it appears that Bjerrum and Gjaldbaek's "reaction constant" for calcium carbonate can be used without conversion if we confine ourselves to the experiments they signify as best suited for the calculation (0·1 and 0·02n $CaCl_2$), because a recalculation using 0·46 instead of 0·30 gives no difference in the second decimal place. Calculated by (144) p_{K_1} becomes

$$p_{K_1} = 6{\cdot}515 \text{ at } 18°,$$

which is in surprisingly good agreement with Walker and Cormack's, Kendall's and my own determinations.

At 18° the first dissociation exponent is therefore according to

Knox	6·426	Kendall	6·507
Walker and Cormack	6·512	Bjerrum and Gjaldbaek	6·515
Hasselbalch	6·420	Warburg[2]	6·514

Of these values Knox's was obtained with a relatively indifferent technique. In view of what was said in chapter IV Hasselbalch's experiments may very well have a systematic error; all the other determinations give values which approximate closely to 6·51. Therefore

$$K_1 = 3{\cdot}1 \times 10^{-7} \ (18°).$$

T. H. Milroy [1917] has carried out electrical determinations at 37·5° with pure 0·2n sodium bicarbonate solution and the same diluted with 0·2n sodium chloride solution from which $p_{K'_1}$ at the concentration 0·2n can be calculated.

[1] Cited from Bjerrum and Gjaldbaek [1919].

[2] Sodium salts.

I have gone over Milroy's experiments and the technique is hardly as good as could be wished. The dispersion is rather large and $p_{K'_1}$ has a lower value than would be expected, being not as much as 6·00 on an average. J. F. McClendon, A. Shedlov and W. Thomson [1917] have also made measurements in salt solutions containing bicarbonate but they have only published their results graphically so that it is impossible to adjudicate upon the accuracy of the determinations.

Lastly L. Michaelis and P. Rona [1914] have made some measurements in sodium bicarbonate solutions but as Hasselbalch has shown they are subject to a technical error which makes them very doubtful.

The osmotic coefficient of the bicarbonate can be calculated with equation (44),

$$1 - f_0 = k \sqrt[3]{c},$$

where k is determined by (46),

$$\log_e f_a = -4k \sqrt[3]{c}.$$

By substituting the value 2·303 × 0·46 for $4k$ we get

$$f_0 (HCO'_3) = 1 - 0{\cdot}26 \sqrt[3]{c}. \quad \text{.....(150)}$$

According to (136) $f_\mu (NaHCO_3) = 1 - 0{\cdot}51 \sqrt[3]{c},$

and from (143) we have

$$\log f_a (HCO'_3) = (\log F_a (HCO'_3)) = -0{\cdot}46 \sqrt[3]{c}.$$

With the help of these equations the deviation coefficients for the bicarbonate ion have been calculated and the results recorded in Table XLVI and graphically displayed in Fig. 14.

When one compares the deviation coefficients given here with those valid for KCl which are recorded in chapter I, Table III one cannot help thinking the marked depression of the activity of the bicarbonate ion and the relatively low conductivity coefficient are an indication that the salt is not completely dissociated as we previously supposed.

It would be of great interest to compare the deviation coefficients of a considerable number of salts with one another and with the dissociation constant of the corresponding acid, but such an investigation lies outside the scope of this work and it is to be hoped it will soon be undertaken by someone more expert in that branch of the subject. It may however be put forward that the fact that the activity of the bicarbonate ion can be determined with (143) over a large range of concentration does not at all well fit in with the idea of an incomplete dissociation of sodium bicarbonate. If we assume that equation (143) only represents the relation between the bicarbonate concentration and the activity of its ions, while the relation between the ionic concentration and activity of bicarbonate and sodium is given by

$$-\log F_a = 0{\cdot}25 \sqrt[3]{c}, \quad \text{.....(I)}$$

we can construct the following equation:

$$c\gamma \,.\, 10^{-0{\cdot}25 \sqrt[3]{c\gamma}} = c \,.\, 10^{-0{\cdot}46 \sqrt[3]{c}}, \quad \text{.....(II)}$$

and therefore

$$\log \gamma = \sqrt[3]{c} \,(0{\cdot}46 - 0{\cdot}25 \sqrt[3]{\gamma}). \quad \text{.....(III)}$$

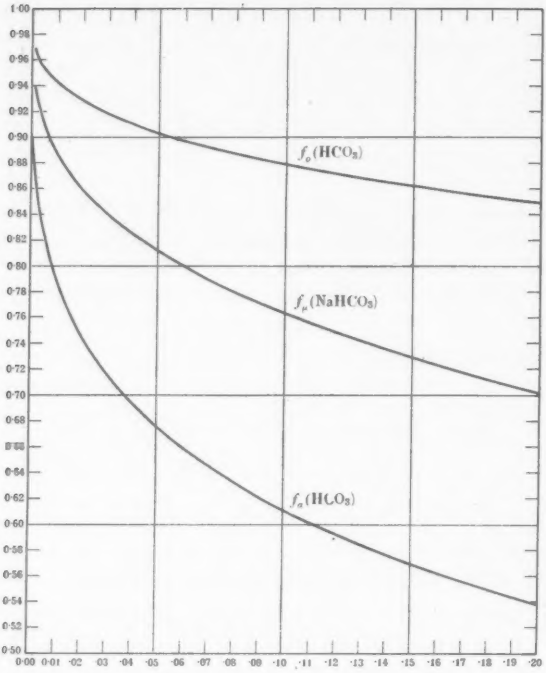


Fig. 14. Deviation coefficients of sodium bicarbonate solutions.

According to the mass action law the following holds:

$$\frac{C_{Na^+} \cdot F_a(Na^+) \cdot C_{HCO'_3} F_a(HCO'_3)}{C_{NaHCO_3} F_a(NaHCO_3)} = K. \quad \text{.....(IV)}$$

If we put $F_a(NaHCO_3) = 1$ we get

$$\frac{c\gamma \cdot 10^{-0{\cdot}5 \sqrt{3}/c\gamma}}{1-\gamma} = K. \quad \text{.....(V)}$$

If now equation (III) is solved by successive approximations and the values found are substituted in (V) we obtain

c	γ	K
0·001	0·952	$1{\cdot}7 \times 10^{-2}$
0·01	0·897	$6{\cdot}1 \times 10^{-2}$
0·1	0·790	$1{\cdot}8 \times 10^{-1}$

The example given shows therefore that "the constant" in (V) must increase with the salt concentration and that the mass action law cannot therefore be satisfied if the salt is incompletely dissociated, provided that an equation for the relation between the concentration and ionic activity of the form of (143) holds good.

In Table XLVII are given determinations of $p_{K'_1}$ in solutions which contain small amounts of phosphate in addition to NaCl and $NaHCO_3$. It

is probable that $p_{K'_1}$ allowing for experimental error is identical with the constant one might expect if phosphate was substituted by chloride.

In Table XLVIII are recorded two series of estimations carried out in about 0·01*n* sodium bicarbonate solutions which were at the same time 0·501*m* as regards cane sugar.

The solutions were prepared by making stock solutions double the strength of those used in the experiments. These were kept on ice. Equal parts of sodium bicarbonate and cane sugar solutions were measured with Geissler pipettes and mixed half an hour before saturation was commenced during which time they were in the laboratory at room temperature.

The potential established itself—in contrast to the pure salt solutions—not immediately, but only when the electrode had been rocked 10–25 times. The first series of measurements belongs to series A and was performed late in this series so that $p_{K'_1}$ is too low. It shows that the relative absorption coefficient is about 0·835 and y is therefore 16·5. The second series of measurements was carried out with E III and E VI simultaneously with the experiments previously noted as technically the best. It gives $p_{K'_1} = 6{\cdot}461$ (18°). As $p_{K'_1}$ in the corresponding pure $NaHCO_3$ solution is 6·413, it appears that the addition of cane sugar increases the constant. If now we regard the depression of solubility of CO_2 as an expression of the amount of water the cane sugar has appropriated (we assume that CO_2 and $NaHCO_3$ are insoluble in hydrated cane sugar), and correct the calculations for this we obtain

$$p_{K'_1} - \log F_a\,(CO_2) = 6{\cdot}383,$$

and at the same time the solution may be regarded as 0·012*n* with respect to bicarbonate, which corresponds to

$$p_{K'_1} = 6{\cdot}402.$$

The difference between 6·383 and 6·402 does not lie outside the experimental error for certain, so much the more so because of the peculiarity in the establishment of the potential in cane sugar solutions, just referred to.

The result of this small series of experiments is that the activity of the bicarbonate ion is not affected with any certainty by the cane sugar molecules when a correction for the hydration is made.

Résumé.

I. The first dissociation constant of carbonic acid has been recalculated from the best experiments in the literature, and it has been determined afresh by the electrical potential method at 18° and 38°.

II. The apparent activity coefficient of the bicarbonate ion has been determined in sodium and potassium solutions up to 0·4*n*.

III. The apparent osmotic coefficient of the bicarbonate ion has been calculated from the apparent activity coefficient.

IV. The apparent conductivity coefficient of the bicarbonate ion has been recalculated.

V. It has been rendered probable that the alkaline bicarbonates are completely dissociated.

ADDENDUM

After this chapter was completed a paper by C. Lovatt Evans appeared (*J. Physiol.* 1921, **54**, pp. 353–366) which necessitates a certain amount of criticism. The author himself is rather cautious, as he writes: "Although perhaps some doubt still remains as to the finality of the conclusions which will be presented here etc...."

The object of the paper was to prove that electrical determinations of the reaction of bicarbonate solutions give too acid values (too low $p_{H'}$) and that correct results can be obtained by the colorimetric method. The explanation of the reaction being found too acid by potential measurements is given by the author as a consequence of the formation of formic acid in sufficient quantity to set up a reduction potential by the catalytic action of the platinum black on the electrode, according to the equations

$$CO_2 + H_2 \rightleftharpoons HCOOH$$

and

$$HCO'_3 + H_2 = HCOO' + H_2O.$$

(1) It can very easily be shown that the combined CO_2 in an alkali bicarbonate solution does not decrease when the solution is treated for half an hour (or one hour) with hydrogen in the presence of platinum black. I have myself carried out over 50 such experiments. However it would be very probable that the combined amount of CO_2 would decrease if a reduction potential was set up as Evans suggests.

(2) If such a process took place $p_{K'_1}$ would be dependent upon the CO_2 tension and HCO'_3 concentration and not, as has been shown, practically only upon the cation concentration.

(3) The calculation of p_{K_1} from Bjerrum and Gjaldbaek's and from my own experiments would give lower values than from conductivity determinations if the formation of formic acid took place.

(4) The agreement between the calculated amount of combined CO_2 and that found experimentally in the phosphate experiments in chapter XI would be bad if Evans was right in his conclusions.

The most important of Evans' experiments are the following ($p_{K'_1}$ calculated by the author):

mm. Hg CO_2	Neutral red		Phenol red		Electrically	
	$p_{H'}$	pK'_1	$p_{H'}$	pK'_1	$p_{H'}$	pK'_1
			0·02*n* $NaHCO_3$ 20°			
8·93	8·23	(6·59)	8·04	6·40	7·96	(6·33)
21·8	7·70	6·45	7·42	6·17	7·55	6·30
33·3	7·57	6·51	7·28	6·22	7·34	6·28
46·0	7·37	6·45	7·17	6·25	7·16	6·24
68·9	7·19	6·34	6·97	6·12	7·07	6·22
		Mean 6·44		Mean 6·19		Mean 6·26
			0·02*n* $NaHCO_3$ + 0·18*n* NaCl 20°			
6·5	8·13	(6·36)	—	—	—	—
11·5	7·77	6·25	—	—	—	—
13·9	7·66	6·22	7·67	6·23	7·60	6·16
18·8	—	—	—	—	7·34	6·06
26·0	7·48	6·31	—	—	—	—
30·5	7·33	6·23	7·34	6·24	7·15	6·05
42·8	7·23	6·28	—	—	—	—
44·0	7·21	6·27	—	—	—	—
46·1	—	—	—	—	7·08	6·16
57·2	7·11	6·28	—	—	—	—
58·5	—	—	—	—	6·88	6·10
65·4	7·04	6·27	—	—	—	—
		Mean 6·26		Mean 6·235		Mean 6·11

From the determinations made in the preceding chapter $p_{K'_1}$ in the first series is 6·38 and in the second series 6·23 at 20°.

The reason Evans did not obtain agreement between the electrical and colorimetric results is probably the fact that his electrode vessel is of an unsuitable type. As far as I can judge it must be almost impossible with his electrode to avoid the O_2 error. Evans himself writes: "Definitive potentials were obtained almost at once provided the gas was free from oxygen; when this was not the case, there was first a somewhat lower potential than that finally obtained after one or two hundred inversions."

The colorimetric determinations of the reaction in salt solutions seem from the above to be rather good (agreement between $p_{K'_1}$ in each series), but the curves of the reaction of the blood indicate too alkaline a reaction possibly because the blood was saturated at body temperature, the dialysis and colorimetry however being performed at room temperature.

The reactions of salt solutions which are "saturated" with smaller CO_2 tensions than 10 mm. are more alkaline than would be expected as Evans himself has noted. He ascribes this to hydrolysis which is incorrect as hydrolysis at the reactions of the experiments is negligible in this connection (it is of the same order as $a_{OH'}$). The strongly alkaline reactions probably arise from equilibrium not being reached during the "saturation" at the low CO_2 tensions (cf. chapter XI).

I shall not enter further into a discussion of the theory of the "degree of dissociation" of the bicarbonate here but refer the reader to the preceding chapters.

Table XXX. Conductivity determinations in bicarbonate solutions.

V	c	λ/c	μ_v	
12·1	0·0826	0·436	77·3	Kendall
24·2	0·0413	0·346	81·7	$NaHCO_3$ 25°
48·4	0·0207	0·275	85·3	
96·8	0·0103	0·217	88·2	
193·6	0·00516	0·173	90·6	
387·2	0·00258	0·137	92·4	
774·4	0·00129	0·109	93·8	
1548·8	0·000646	0·0864	94·8	
3097·6	0·000323	0·0686	95·5	
—	0	0	99·2	
32·0	0·0313	0·315	69·8	Walker and Cormack
64·0	0·0156	0·250	72·9	$NaHCO_3$ 18°
128·0	0·00782	0·198	75·6	
256·0	0·00391	0·158	78·6	
518·0	0·00195	0·125	80·8	
—	0	0	88·1	
64·0	0·0156	0·250	83·3	Kendall
128·0	0·00782	0·198	88·8	1/2 $CaCO_3$ 25°
256·0	0·00391	0·158	93·4	
512·0	0·00195	0·125	96·7	
1024·0	0·000977	0·0992	102·0	
2048·0	0·000488	0·0787	107·7	
—	0	0	?	

Table XXXI. Determinations of the first dissociation constant of carbonic acid calculated from Walker and Cormack's and from Kendall's experiments.

V	μ_v	γ	$K_1 \times 10^{-7}$	
31·25	1·104	0·00310	3·09	Walker and Cormack
62·5	1·570	0·00441	3·13	18° CO_2 from marble
93·7	1·916	0·00539	3·11	
125·0	2·218	0·00623	3·12	
∞	355·8		Mean 3·11	
			p_{K_1} 6·507	
27·5	1·033	0·00291	3·07	Walker and Cormack
55·0	1·454	0·00409	3·05	18° CO_2 from carbonic acid snow
82·5	1·754	0·00502	3·05	
110·0	2·052	0·00577	3·04	
∞	355·8		Mean 3·05	
			p_{K_1} 6·516	
25·4	0·631	0·00237	2·23	Kendall
38·3	0·770	0·00290	2·20	0°
50·0	0·880	0·00331	2·20	
76·3	1·081	0·00407	2·18	
99·8	1·242	0·00468	2·21	
152·6	1·548	0·00584	2·24	
∞	265·6		Mean 2·21	
			p_{K_1} 6·656	
30·9	1·100	0·00309	3·11	Kendall
42·0	1·281	0·00360	3·11	18°
61·2	1·550	0·00437	3·14	
83·4	1·792	0·00504	3·06	
∞	355·8		Mean 3·11	
			p_{K_1} 6·507	
36·4	1·403	0·00355	3·47	Kendall
51·3	1·659	0·00320	3·44	25°
72·8	1·977	0·00500	3·45	
102·4	2·341	0·00592	3·44	
145·5	2·820	0·00713	3·54	
∞	395·5		Mean 3·47	
			p_{K_1} 6·460	

Table XXXII. Calculation of $p_{K'_1}$ in pure sodium bicarbonate solutions from K. A. Hasselbalch's experiments, *Biochem. Zeitsch.* **78**, p. 119.

		Uncorrected		Corrected for decreased H_2 pressure	
Concentration	mm. CO_2	p_H	$p_{K'_1}$	p_H	$p_{K'_1}$
0·05n	47·2	7·47	6·19	7·48	6·20
$^g/_c = 0·368$	403·2	6·53	6·18	6·68	(6·33)[1]
18°	74·0	7·28	6·20	7·30	6·22
Mark ♁	147·7	6·97	6·19	7·01	6·23
		Mean	6·19	Mean	6·22
0·01n	54·3	6·82	6·29	6·83	6·30
$^g/_c = 0·215$	134·3	6·42	6·28	6·46	6·32
18°	101·8	6·51	6·25	6·54	6·29
Mark ♁	84·5	6·59	6·25	6·61	6·27
	68·8	6·73	6·30	6·75	6·32
	92·4	6·59	6·29	6·62	6·32
		Mean	6·28	Mean	6·30

[1] Not included in the mean value.

Table XXXII (*continued*)

Concentration	mm. CO_2	$p_{H'}$ Uncorrected	pK'_1 Uncorrected	$p_{H'}$ Corrected for decreased H_2 pressure	pK'_1 Corrected for decreased H_2 pressure
0·05*n*	168·9	7·02	6·06	7·08	6·12
$\sqrt[3]{c}$=0·368	247·8	6·87	6·08	6·97	(6·18)[1]
38°	52·6	7·55	6·09	7·57	6·11
Mark ♀	717·0	6·40	6·07	?	?
		Mean	6·08	Mean	6·12
0·03*n*	40·6	7·48	6·12	7·49	6·13
$\sqrt[3]{c}$=0·311	80·7	7·15	6·09	7·18	6·12
38°	135·0	6·92	6·09	6·96	6·13
Mark ♀	96·9	7·08	6·10	7·11	6·13
		Mean	6·10	Mean	6·13
0·02*n*	123·3	6·83	6·13	6·87	6·17
$\sqrt[3]{c}$=0·271	36·7	7·33	6·11	7·34	6·12
38°	62·8	7·13	6·14	7·15	6·16
Mark ♀		Mean	6·13	Mean	6·15
0·01*n*	85·0	6·70	6·14	6·73	6·17
$\sqrt[3]{c}$=0·215	218·6	6·33	6·18	6·40	6·25
38°	137·4	6·51	6·16	6·55	6·20
Mark ♀	189·2	6·40	6·19	6·46	6·23
	119·8	6·57	6·16	6·61	6·20
	149·0	6·44	6·13	6·48	6·17
		Mean	6·16	Mean	6·20
0·005*n*	23·5	7·02	6·21	7·03	6·22
$\sqrt[3]{c}$=0·171	41·35	6·75	6·18	6·76	6·19
Mark ♀	71·8	6·53	6·20	6·55	6·22
		Mean	6·20	Mean	6·21

[1] Not included in the mean value.

Table XXXIII. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions carried out with the small saturator electrode. Series A. Mark △.

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	$p_{H'}$	$p_{K'_1}$ 18°	
T=8·88	16·0	240·2	0·6	5·84	6·37	
0·00399*n* HCO'_3	16·5	118·6	0·2	6·19	6·42	
0·00399*n* Na'	16·5	41·6	0·2	6·56	6·33	
	16·5	15·2	0·2	7·04	6·37	
	16·5	5·8	0·2	7·45	6·37	
				Mean	6·37	
T=17·52	19·5	543·4	0·3	5·80	(6·37)	
0·00786*n* HCO'_3	19·5	141·0	0·5	6·38	6·37	
0·00786*n* Na'	19·5	55·2	0·5	6·82	6·40	
	19·5	18·0	0·5	7·29	6·38	
	20·0	7·1	0·3	7·70	6·39	
	20·0	4·2	0·3	8·05	(6·51)	
				Mean	6·38	
T=55·5	19·5	486·5	0·7	6·35	6·37	
0·0249*n* HCO'_3	19·0	124·4	0·5	6·95	6·38	
0·0249*n* Na'	19·0	51·0	0·5	7·33	6·37	$\sqrt[3]{c}$=0·273
	19·0	30·9	0·3	7·51	6·35	
	19·0	11·3	0·3	8·01	6·40	
				Mean	6·37	

Table XXXIII (*continued*)

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°	
$T=110{\cdot}0$	20·5	538·0	0·6	6·60	(6·37)	
0·0494n HCO'_3	20·0	267·0	0·5	6·91	6·37	
0·0494n Na^+	20·0	67·2	0·5	7·50	6·36	$\sqrt[3]{c}=0{\cdot}367$
	20·0	25·2	0·2	7·91	6·35	
	20·0	9·2	0·2	8·36	6·37	
				Mean	6·36	
$T=221{\cdot}9$	19·5	126·3	0·5	7·44	6·28	
0·0997n HCO'_3	19·5	37·5	0·5	8·00	6·32	
0·0997n Na^+	19·5	14·7	0·3	8·39	6·31	
	19·0	58·8	0·3	7·80	6·31	
	17·0	193·4	0·2	7·28	6·31	$\sqrt[3]{c}=0{\cdot}464$
	17·0	85·3	0·9	7·63	6·31	
	17·0	25·1	1·2	8·08	6·33	
	17·0	50·1	0·7	7·88	6·32	
	17·0	130·1	0·5	7·41	6·27	
	17·0	56·6	0·3	7·81	6·32	
				Mean	6·31	

Table XXXIV. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of 0·42 % NaCl (0·0718n). Carried out with the small saturator electrode. Series A. Mark △.

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°	
$T=8{\cdot}88$	18·5	245·8	0·6	5·78	6·31	
0·00399n HCO'_3	19·0	29·5	0·6	6·70	6·31	
0·0718n Cl'	19·0	40·7	0·6	6·56	6·30	$\sqrt[3]{c}=0{\cdot}421$
0·07479n Na^+	19·0	63·8	0·4	6·23	6·28	
				Mean	6·30	
$T=17{\cdot}52$	19·0	395·5	1·0	5·86	6·31	
0·00786n HCO'_3	19·0	108·9	0·4	6·42	6·28	
0·0718n Cl'	19·5	34·5	0·2	6·91	6·29	$\sqrt[3]{c}=0{\cdot}429$
0·07966n Na^+	19·5	6·0	0·2	7·68	6·30	
	19·5	14·4	0·2	7·31	(6·31)	
				Mean	6·29	
$T=55{\cdot}5$	20·0	36·7	6·6	7·34	(6·24)	
0·0249n HCO'_3	20·0	119·2	1·2	6·86	6·27	
0·0718n Cl'	20·0	106·2	0·7	6·93	6·29	
0·0967n Na^+	20·0	7·3	0·5	8·10	6·30	$\sqrt[3]{c}=0{\cdot}459$
	20·0	512·0	0·6	6·22	(6·25)	
	20·0	126·7	0·3	6·85	6·29	
	20·0	36·1	0·2	7·40	6·29	
	20·0	3·1	0·2	8·46	(6·31)	
				Mean	6·29	
$T=110{\cdot}0$	21·0	144·2	0·2	7·10	6·29	
0·0494n HCO'_3	21·0	48·3	0·2	7·57	6·29	
0·0718n Cl'	21·0	34·7	0·2	7·72	6·29	$\sqrt[3]{c}=0{\cdot}495$
0·1212n Na^+	20·5	7·7	0·2	8·36	6·29	
	20·5	3·8	0·2	8·77	(6·43)	
				Mean	6·29	
$T=221{\cdot}9$	17·0	476·4	1·5	6·76	(6·18)	
0·0997n HCO'_3	17·5	122·5	0·8	7·41	6·23	
0·0718n Cl'	17·0	58·2	0·3	7·77	6·28	$\sqrt[3]{c}=0{\cdot}556$
0·1715n Na^+	17·0	23·3	0·3	8·15	6·27	
	17·5	22·2	0·3	8·17	6·27	
				Mean	6·26	

Table XXXV. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of 0·85 % sodium chloride (0·145*n*). Carried out with the small saturator electrode. Series A. Mark △.

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°	
$T = 8·88$	19·0	238·4	0·9	5·78	6·30	
0·00399*n* HCO'_3	19·0	111·6	0·0	6·10	6·29	
0·145*n* Cl′	19·0	30·1	0·3	6·64	6·26	$\sqrt[3]{c} = 0·530$
0·1490*n* $Na^·$	19·0	12·2	0·3	7·03	6·26	
	19·0	63·7	0·3	6·33	6·27	
	19·0	12·6	0·3	7·02	6·27	
				Mean	6·28	
$T = 17·52$	20·0	587·6	0·7	5·60	(6·20)	
0·00786*n* HCO'_3	19·5	149·5	0·3	6·24	6·26	
0·145*n* Cl′	19·0	43·9	0·5	6·76	6·24	$\sqrt[3]{c} = 0·535$
0·1529*n* $Na^·$	19·0	14·3	0·4	7·23	6·22	
	19·0	6·3	0·4	7·61	6·24	
	19·0	3·6	0·4	7·87	(6·26)	
				Mean	6·24	
$T = 55·5$	21·0	152·0	0·4	6·72	6·22	
0·0249*n* HCO'_3	20·5	47·6	0·4	7·24	6·24	
0·145*n* Cl′	21·0	14·6	1·6	7·77	6·26	$\sqrt[3]{c} = 0·554$
0·1699*n* $Na^·$	20·5	2·7	0·4	8·55	(6·25)	
	19·5	598·0	1·1	6·13	(6·24)	
	19·5	411·7	0·5	6·27	6·22	
				Mean	6·24	
$T = 110·0$	19·5	60·9	0·2	7·44	6·27	
0·0494*n* HCO'_3	19·5	26·5	0·2	7·81	6·28	
0·145*n* Cl′	19·5	7·7	0·2	8·34	6·29	
0·1844*n* $Na^·$	20·5	565·0	1·4	6·42	(6·25)	$\sqrt[3]{c} = 0·569$
	20·5	142·0	0·6	7·06	6·23	
	20·5	27·7	0·6	7·79	6·28	
				Mean	6·27	
$T = 221·9$	17·0	460·5	0·6	6·73	(6·14)	
0·0997*n* HCO'_3	17·0	161·2	0·6	7·24	6·19	
0·0997*n* Cl′	17·0	49·1	0·2	7·79	6·23	
0·2447*n* $Na^·$	17·0	15·5	0·2	8·28	6·23	$\sqrt[3]{c} = 0·625$
	17·0	67·9	0·2	8·64	6·25	
	19·0	45·0	0·6	7·83	6·22	
	19·0	90·9	0·7	7·56	6·26	
	19·0	32·8	0·6	8·00	6·25	
				Mean	6·23	

Table XXXVI. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of 1·7 % NaCl (0·291*n*). Carried out with the small saturator electrode. Series A. Mark △.

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°	
$T = 8·88$	18·5	243·4	0·4	5·69	6·22	
0·00399*n* HCO'_3	19·0	115·5	0·4	6·00	6·20	
0·291*n* Cl′	19·0	36·1	0·4	6·52	6·22	$\sqrt[3]{c} = 0·666$
0·2950*n* $Na^·$	19·5	11·7	0·4	7·00	6·21	
	19·5	16·1	0·4	6·85	6·19	
				Mean	6·21	
$T = 17·52$	18·5	529·3	1·1	5·57	(6·13)	
0·00786*n* HCO'_3	18·5	149·5	0·8	6·17	6·19	
0·291*n* Cl′	18·5	46·2	0·2	6·67	6·18	$\sqrt[3]{c} = 0·669$
0·2989*n* $Na^·$	19·0	15·3	0·2	7·17	6·19	
				Mean	6·19	

Table XXXVI (*continued*)

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°	
$T=55{\cdot}5$	19·5	602·7	1·1	6·08	(6·20)	
0·0249n HCO'_3	19·5	416·2	0·5	6·27	6·23	
0·291n Cl'	19·5	131·1	0·6	6·73	6·19	$\sqrt{z}/c=0{\cdot}681$
0·3159n Na^+	19·0	62·0	0·5	7·09	6·23	
	19·0	17·2	0·5	7·61	6·18	
	19·0	6·0	0·5	8·08	6·19	
	19·0	2·8	0·5	8·48	(6·19)	
				Mean	6·20	
$T=110{\cdot}0$	20·0	518·9	0·4	6·50	(6·25)	
0·0499n HCO'_3	20·0	139·1	0·4	7·01	6·19	
0·291n Cl'	20·5	48·4	0·4	7·50	6·21	$\sqrt{z}/c=0{\cdot}699$
0·3409n Na^+	20·0	16·2	0·4	7·94	6·19	
				Mean	6·19	
$T=221{\cdot}9$	17·0	442·1	0·6	6·76	6·15	
0·0997n HCO'_3	17·0	40·0	0·4	7·84	6·19	
0·291n Cl'	17·0	20·7	0·4	8·09	6·15	
0·3907n Na^+	17·0	40·1	0·4	7·83	6·18	
	17·0	14·5	0·4	8·23	6·14	$\sqrt{z}/c=0{\cdot}731$
	18·0	95·2	0·6	7·50	6·18	
	18·0	106·4	0·6	7·39	6·15	
	18·0	365·2	0·7	6·86	6·17	
	18·0	26·1	0·4	8·04	6·19	
				Mean	6·17	

Table XXXVII. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of varying quantities of KCl. Carried out with the small saturator electrode. Series A.

Concentration	Temp.	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 18°
$T=44{\cdot}3$	20·0	80·4	0·3	6·98	6·32
0·0199n HCO'_3	20·0	132·0	0·3	6·79	6·34
0·54 % KCl (0·0718n)	20·0	15·4	0·6	7·71	6·33
				Mean	6·33
$T=110{\cdot}0$	18·0	58·6	0·5	7·48	6·28
0·0494n HCO'_3	18·0	98·7	1·7	7·26	6·29
0·54 % KCl (0·0718n)	18·0	21·2	0·5	7·92	6·29
				Mean	6·29
$T=44{\cdot}3$	19·0	130·3	0·6	6·72	6·27
0·0199n HCO'_3	19·0	37·1	0·8	7·27	6·28
1·08 % KCl (0·145n)	19·5	13·8	0·6	7·69	6·26
	19·5	56·2	0·6	7·09	6·27
				Mean	6·27
$T=44{\cdot}3$	19·0	64·3	0·5	6·98	6·22
0·0199n HCO'_3	20·0	95·3	0·7	6·84	6·24
2·16 % KCl (0·291n)	20·0	30·1	0·7	7·34	6·25
	19·5	39·3	0·7	7·20	6·23
				Mean	6·24
$T=55{\cdot}5$	17·0	132·2	0·5	6·76	6·20
0·0249n HCO'_3	17·0	55·0	0·4	7·14	6·23
2·16 % KCl (0·291n)	17·0	18·1	0·6	7·61	6·22
				Mean	6·22
$T=111{\cdot}0$	18·0	58·6	0·6	7·40	6·21
0·0494n HCO'_3	18·0	98·7	1·7	7·17	6·21
2·16 % KCl (0·291n)	18·0	21·2	0·5	7·85	6·22
				Mean	6·21

Table XXXVIII. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of 0·145*n* NaCl or KCl. Carried out with the small saturator electrode. Series A.

		mm. Hg		NaCl		KCl	
Concentration	Temp.	CO_2	O_2	p_H	pK'_1	p_H	pK'_1 18°
$T = 56·25$	17·0	162·2	0·2	6·66	6·21	6·69	6·24
0·0253*n* HCO'_3	17·5	59·1	0·7	7·11	6·23	7·16	6·28
					Mean 6·22		Mean 6·26
$T = 111·0$	17·0	125·3	0·4	7·07	6·21	7·10	6·25
0·0499*n* HCO'_3	17·5	34·2	1·7	7·68	6·23	7·70	6·28
	17·5	45·3	0·4	7·54	6·24	7·57	6·28
					Mean 6·23		Mean 6·27

Table XXXIX. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with varying additions of salt. Series A.

		0·0718*n*		0·0145*n*		0·291*n*	
T	No salt added	NaCl	KCl	NaCl	KCl	NaCl	KCl
8·88	(6·37)	6·30	—	6·28	—	6·21	—
17·5	(6·38)	6·29	—	6·24	—	6·19	—
44·3	—	—	6·33	—	6·27	—	6·24
55·5	6·37	6·29	—	6·24	—	6·20	6·22
110·0	6·36	6·29	6·29	6·27	6·27	6·19	—
111·0	—	—	—	—	6·23	—	6·21
221·9	6·31	6·26	—	6·23	—	6·17	—

Table XL. Determinations of $p_{K'_1}$ for 18° in sodium bicarbonate solutions with the addition of salt. Carried out with the small saturator electrode. Series B. Mark +.

		mm. Hg			
Concentration	Temp.	CO_2	p_H	pK'_1 18°	
$T = 45·2$	21·0	50·1	7·18	6·30	
0·0203*n* HCO'_3	21·0	8·7	7·90	6·26	$\sqrt[3]{c} = 0·452$
0·42 % NaCl (0·0718*n*)	21·0	27·7	7·46	6·32	
0·0718*n* Cl'				Mean 6·29	
0·0921*n* Na^+					
$T = 56·5$	15·0	579·4	6·18	6·32	
0·0254*n* HCO'_3	15·5	139·0	6·79	6·31	
0·42 % NaCl (0·0718*n*)	15·5	30·1	7·50	6·35	$\sqrt[3]{c} = 0·458$
0·0718*n* Cl'	19·5	61·8	7·20	6·34	
0·0962*n* Na^+	19·5	52·0	7·27	6·32	
	19·5	41·6	7·39	6·35	
	20·0	41·6	7·38	6·33	
				Mean 6·33	
$T = 45·2$	21·0	51·2	7·16	6·30	
0·0203*n* HCO'_3	21·0	36·0	7·30	6·28	$\sqrt[3]{c} = 0·452$
0·42 % NaCl (0·0718*n*)				Mean 6·29	
0·0718*n* Cl'					
0·0921*n* Na^+					
$T = 55·7$	20·0	36·5	7·42	6·30	
0·0250*n* HCO'_3	20·0	23·2	7·55	6·24	
0·85 % NaCl (0·145*n*)	20·0	22·2	7·60	6·27	$\sqrt[3]{c} = 0·554$
0·145*n* Cl'	21·0	38·4	7·36	6·26	
0·170*n* Na^+	19·0	142·6	7·76	6·24	
	19·0	53·2	7·22	6·27	
				Mean 6·26	

Table XL (*continued*)

Concentration	Temp.	mm. Hg CO_2	p_H	$p_{K'_1}$ 18°	
$T=56{\cdot}5$	21·5	579·4	6·13	(6·22)	
0·0254n HCO'_3	21·0	111·3	6·92	6·29	
0·85 % NaCl (0·145n)	21·0	48·5	7·26	6·27	$\sqrt[3]{c}=0{\cdot}558$
0·145n Cl'	21·0	31·8	7·46	6·29	
0·174n $Na^{\cdot}$	21·0	49·0	7·26	6·26	
	21·0	19·7	7·68	6·30	
			Mean	6·28	
$T=56{\cdot}5$	19·5	199·8	6·55	6·19	
0·0254n HCO'_3	19·0	81·3	6·97	6·21	
1·7 % NaCl (0·291n)	19·0	36·8	7·29	6·18	$\sqrt[3]{c}=0{\cdot}681$
0·291n Cl'	19·0	20·0	7·58	6·21	
0·3164n $Na^{\cdot}$	19·0	8·4	7·96	6·22	
	19·0	17·1	7·65	6·21	
			Mean	6·20	
$T=56{\cdot}5$	19·5	61·8	7·18	6·30	
0·025n HCO'_3	19·5	52·5	7·25	6·31	$\sqrt[3]{c}=0{\cdot}554$
1·08 % KCl (0·145n)	19·5	41·6	7·34	6·30	
0·145n Cl'	20·0	41·6	7·36	6·30	
0·145n $K^{\cdot}$					
0·025n $Na^{\cdot}$			Mean	6·30	

Table XLI. Determinations of $p_{K'_1}$ in salt solutions for 18°. Carried out by Höber technique in E VI and E III. Series B. Mark ○.

Concentration	Temp.	mm. Hg CO_2	p_H	$p_{K'_1}$ 18° E VI	$p_{K'_1}$ 18° E III	
$T=224{\cdot}4$	19·0	96·2	7·533	6·247	—	$n/10$ $NaHCO_3$
0·1008n HCO'_3	19·0	65·9	7·737	6·287	—	
0·1008n $Na^{\cdot}$	19·0	40·7	7·969	6·299	—	
	19·0	31·6	8·051	6·281	—	$\sqrt[3]{c}=0{\cdot}464$
	19·0	31·6	8·061	6·292	—	
	19·0	30·7	8·070	6·288	—	
	19·0	32·7	8·026	6·261	—	
			Mean	6·279		
$T=112{\cdot}2$	18·0	86·9	7·364	6·340	—	$n/20$ $NaHCO_3$
0·05041n HCO'_3	18·0	86·9	7·364	—	6·340	
0·05041n $Na^{\cdot}$	18·0	61·5	7·506	6·321	—	
	18·0	61·5	7·497	—	6·323	$\sqrt[3]{c}=0{\cdot}369$
	18·0	50·7	7·604	6·346	—	
	18·0	50·7	7·597	—	6·349	
			Mean	6·335	6·337	
			„	6·335		
$T=56{\cdot}49$	18·5	52·7	7·324	6·384	—	$n/40$ $NaHCO_3$
0·02538n HCO'_3	20·0	24·8	7·680	6·400	—	
0·02538n $Na^{\cdot}$	20·0	14·7	7·890	6·383	—	$\sqrt[3]{c}=0{\cdot}294$
	20·0	32·0	7·571	6·402	—	
	19·0	47·8	7·382	6·391	—	
			Mean	6·393		
$T=22{\cdot}96$	17·0	104·1	6·644	6·392	—	$n/100$ $NaHCO_3$
0·01031n HCO'_3	17·0	104·1	6·636	—	6·385	
0·01031n $Na^{\cdot}$	16·5	43·4	7·038	6·416	—	
	16·5	43·4	7·035	—	6·413	$\sqrt[3]{c}=0{\cdot}217$
	19·5	82·2	6·742	6·382	—	
	19·5	35·9	7·133	6·413	—	
	19·5	35·9	7·133	—	6·413	
	19·5	37·3	7·090	6·386	—	
			Mean	6·398	6·403	
			„	6·400		

Table XLI (*continued*)

Concentration	Temp.	mm. Hg CO_2	p_H	pK'_1 18° E VI	pK'_1 18° E III	
$T=111{\cdot}4$	21·0	28·2	7·803	6·291	—	$n/20$ $KHCO_3$
0·05004n HCO'_3	20·5	33·3	7·751	6·308	—	
0·0497n Cl′	20·5	33·3	7·742	—	6·299	$n/20$ KCl
0·1000n $K^{\cdot}$	20·5	49·8	7·600	6·332	—	
	20·5	49·8	7·593	—	6·323	$\sqrt[3]{c}=0{\cdot}464$
	22·0	26·2	7·876	6·319	—	
			Mean	6·313	6·312	Mark ⊗
			,,	6·312		
$T=224{\cdot}4$	20·0	83·3	7·621	6·268	—	$n/10$ $NaHCO_3$
0·1008n HCO'_3	20·0	83·3	7·618	—	6·265	
0·1012n Cl′	20·0	41·1	7·911	6·250	—	$n/10$ KCl
0·1008n $Na^{\cdot}$	20·0	41·1	7·911	—	6·260	
0·1012n $K^{\cdot}$						$\sqrt[3]{c}=0{\cdot}585$
0·09965n Cl′	22·0	91·7	7·586	6·269	—	
0·09965n $K^{\cdot}$	22·0	38·3	7·938	6·242	—	
	22·0	38·3	7·931	—	6·235	
$T=222{\cdot}7$	21·5	85·1	7·611	6·271	—	$n/10$ $KHCO_3$
0·1000n HCO'_3	21·5	85·1	7·604	—	6·264	
0·1012n Cl′	21·5	44·5	7·896	6·275	—	$n/10$ NaCl
0·1000n $K^{\cdot}$						
0·1012n $Na^{\cdot}$			Mean	6·262	6·256	
			,,	6·260		
$T=2{\cdot}244$	19·5	35·2	6·029	6·310	—	
0·001008n HCO'_3	19·5	35·2	6·026	—	6·310	$n/1000$ $NaHCO_3$
0·0994n Cl′	20·0	47·0	5·904	6·303	—	
0·1004n $Na^{\cdot}$	20·0	38·6	5·991	6·298	—	$n/10$ NaCl
	20·0	38·6	5·991	—	6·298	
	19·0	37·4	6·034	6·334	—	
	19·0	37·4	6·031	—	6·333	
	19·5	39·5	5·987	6·318	—	
	19·5	39·5	5·984	—	6·315	
			Mean	6·313	6·314	
			,,	6·313		
$T=221{\cdot}45$	19·5	44·1	7·929	6·322	—	$n/10$ $KHCO_3$
0·09948n HCO'_3	19·5	44·1	7·918	—	6·315	
0·09948n $K^{\cdot}$	19·5	29·2	8·101	6·317	—	
	19·5	29·2	8·094	—	6·310	$\sqrt[3]{c}=0{\cdot}464$
	19·5	60·9	7·799	6·324	—	
	19·5	60·9	7·789	—	6·314	
			Mean	6·321	6·313	Mark ⊗
			,,	6·317		
$T=22{\cdot}14$	19·5	38·9	7·092	6·422	—	
0·009948n HCO'_3	19·5	38·9	7·095	—	6·425	$n/100$ $KHCO_3$
0·009948n $K^{\cdot}$	19·5	48·4	6·979	6·404	—	
	19·5	48·4	6·979	—	6·404	$\sqrt[3]{c}=0{\cdot}215$
	19·5	50·2	6·969	6·410	—	
			Mean	6·412	6·415	Mark ⊗
			,,	6·413		
$T=224{\cdot}4$	20·0	57·8	7·737	6·226	—	$n/10$ $NaHCO_3$
0·1008n HCO'_3	20·0	57·8	7·737	—	6·226	
0·1002n Cl′	19·5	41·5	7·906	6·267	—	$n/10$ NaCl
0·2010n $Na^{\cdot}$	19·5	41·5	7·904	—	6·265	
	22·0	81·3	7·623	6·254	—	
	22·0	81·3	7·621	—	6·252	$\sqrt[3]{c}=0{\cdot}585$
	22·0	29·5	8·048	6·228	—	
	22·0	29·5	8·058	—	6·238	Mark +
	22·5	78·2	7·642	6·249	—	
	22·5	78·2	7·639	—	6·256	
			Mean	6·245	6·247	
			,,	6·246		

Table XLI (*continued*)

Concentration	Temp.	mm. Hg. CO_2	p_H	$p_{K'_1}$ 18° E VI	$p_{K'_1}$ 18° E III	
$T=112{\cdot}2$	21·0	75·6	7·411	6·314	—	$n/20$ $NaHCO_3$
0·05041n HCO'_3	21·0	75·6	7·408	—	6·311	
0·0501n Cl′	22·0	24·3	7·889	6·295	—	$n/20$ NaCl
0·1005n Na^*	22·0	24·3	7·871	—	6·277	
	20·0	31·6	7·773	6·300	—	$\sqrt[3]{c}=0{\cdot}464$
	20·0	31·6	7·761	—	6·288	
			Mean	6·303	6·292	Mark +
			„	6·297		
$T=222{\cdot}7$	21·5	80·9	7·647	6·285	—	$n/10$ $KHCO_3$
0·1000n HCO'_3	21·5	80·9	7·637	—	6·275	$n/10$ KCl
0·0995n Cl′	21·5	29·2	8·070	6·276	—	
0·1995n K^*	21·5	29·2	8·068	—	6·274	$\sqrt[3]{c}=0{\cdot}585$
	21·5	30·4	8·063	6·286	—	
	21·5	30·4	8·063	—	6·296	
			Mean	6·282	6·282	Mark ⊗
			„	6·282		

Table XLII. Determinations of $p_{K'_1}$ at 38°; salt solutions. Carried out by Höber technique in E VI. Series B. Mark o–.

Concentration	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 38°	
$T=56{\cdot}3$	465·7	0·2	6·25	(6·02)	$\sqrt[3]{c}=0{\cdot}681$
0·0253n HCO'_3	108·7	0·2	7·20	6·15	
1·7 % NaCl (0·291n)	44·3	0·2	7·35	6·11	
0·291n Cl′	17·9	0·3	7·22	6·09	
0·3163n Na^*	40·1	0·3	7·38	6·09	
			Mean	6·11	
$T=56{\cdot}5$	587·6	0·0	6·21	(6·09)	$\sqrt[3]{c}=0{\cdot}681$
0·0254n HCO'_3	338·4	0·1	6·45	6·10	
1·7 % NaCl (0·291n)	127·7	0·0	6·89	6·11	
0·291n Cl′	45·8	0·0	7·44	6·11	
0·3164n Na^*	8·7	0·6	7·04	6·08	
			Mean	6·10	

Table XLIII. Determinations of $p_{K'_1}$ at 38° in sodium bicarbonate solutions without the addition of salt. Carried out by Höber technique in E VI. Series B. Mark o–.

Concentration	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 38°	
$T=56{\cdot}5$	54·1	—	7·42	6·27	$\sqrt[3]{c}=0{\cdot}287$
0·0254n HCO'_3	41·4	—	7·56	6·29	
0·0254n Na^*	43·4	—	7·55	6·30	
	95·7	—	7·19	6·28	
	32·1	—	7·66	6·27	
			Mean	6·28	
$T=224{\cdot}4$	74·0	—	7·82	6·20	$\sqrt[3]{c}=0{\cdot}466$
0·101n HCO'_3	59·4	—	7·90	6·18	
0·101n Na^*	90·7	—	7·75	6·22	
	59·5	—	7·87	6·15	
	112·0	—	7·58	6·14	
	50·2	—	7·92	6·14	
			Mean	6·17	

Table XLIV. Determinations of $p_{K'_1}$ at 38° in sodium bicarbonate solutions with the addition of salt. Carried out with the small saturator electrode. Series B. Mark ×.

Concentration	mm. Hg CO_2	mm. Hg O_2	p_H	$p_{K'_1}$ 38°	
$T=45{\cdot}2$	48·8	—	7·31	6·21	$\sqrt{\mu}/c=0{\cdot}452$
0·0203n HCO'_3	8·7	—	8·01	6·17	
0·42 % NaCl (0·0718n)	26·5	—	7·56	6·18	
0·0718n Cl′					
0·0921n $Na^{\cdot}$			Mean	6·18	
$T=56{\cdot}5$	34·6	0·1	7·55	6·19	$\sqrt{\mu}/c=0{\cdot}458$
0·0254n HCO'_3	114·0	0·6	7·02	6·19	
0·42 % NaCl (0·0718n)	52·3	0·3	7·34	6·17	
0·0718n Cl′	39·3	0·0	7·51	6·21	
0·0962n $Na^{\cdot}$			Mean	6·19	
$T=45{\cdot}2$	543·5	—	6·15	(6·09)	$\sqrt{\mu}/c=0{\cdot}549$
0·0203n HCO'_3	62·7	—	7·10	6·11	
0·85 % NaCl (0·145n)	49·2	0·8	7·26	6·17	
0·145n Cl′	36·2	0·5	7·41	6·17	
0·1653n $Na^{\cdot}$	34·4	0·1	7·43	6·18	
	19·1	0·5	7·66	6·15	
			Mean	6·16	
$T=56{\cdot}3$	529·7	0·2	6·28	6·12	$\sqrt{\mu}/c=0{\cdot}554$
0·0253n HCO'_3	107·6	0·2	7·00	6·15	
0·85 % NaCl (0·145n)	37·4	0·2	7·44	6·12	
0·145n Cl′	35·3	0·2	7·47	6·13	
0·1703n $Na^{\cdot}$			Mean	6·13	
$T=56{\cdot}5$	567·3	0·0	6·27	6·14	$\sqrt{\mu}/c=0{\cdot}554$
0·0254n HCO'_3	142·0	0·1	6·89	6·16	
0·85 % NaCl (0·145n)	25·6	0·2	7·61	6·13	
0·145n Cl′	34·3	0·0	7·53	6·18	
0·1704n $Na^{\cdot}$	34·0	0·6	7·54	6·18	
	56·6	0·6	7·30	6·17	
	39·9	0·6	7·45	6·16	
			Mean	6·16	

Table XLV. Conversion of Bjerrum and Gjaldbaek's Table VI.

P_{CO_2}	$c\,10^3$	log K'	log K' converted
0·000504	1·492	−5·619	−5·666
0·000808	1·700	−5·660	−5·698
0·00333	2·744	−5·677	−5·754
0·01387	4·462	−5·688	−5·741
0·0282	5·930	−5·649	−5·707
0·0501	7·200	−5·661	−5·723
0·1422	10·66	−5·635	−5·705
0·2538	13·27	−5·621	−5·697
0·4167	15·75	−5·630	−5·710
0·5533	17·71	−5·613	−5·697
0·7297	19·44	−5·620	−5·705
0·9841	21·72	−5·616	−5·704
	Mean	−5·641	Mean −5·709

Table XLVI. Deviation coefficients of the bicarbonate ion in sodium salt solutions.

C	$\sqrt{\mu}/c$	f_a	f_0	f_μ ($NaHCO_3$)
0·001	0·100	0·900	0·974	0·949
0·005	0·171	0·834	0·955	0·913
0·01	0·215	0·796	0·944	0·890
0·02	0·271	0·751	0·929	0·862
0·03	0·311	0·719	0·919	0·841
0·04	0·342	0·696	0·911	0·827
0·05	0·368	0·677	0·904	0·812
0·06	0·391	0·661	0·898	0·801
0·07	0·412	0·646	0·893	0·790
0·08	0·431	0·634	0·888	0·780
0·09	0·448	0·622	0·884	0·778
0·10	0·464	0·612	0·879	0·764
0·12	0·493	0·593	0·872	0·749
0·14	0·519	0·577	0·865	0·735
0·16	0·543	0·563	0·859	0·723
0·18	0·565	0·550	0·853	0·712
0·20	0·585	0·538	0·848	0·702
0·22	0·604	0·528	0·843	0·692
0·24	0·621	0·518	0·839	0·683
0·25	0·630	0·513	0·836	0·679

Table XLVII. Determinations of $p_{K'_1}$ in salt solutions containing phosphates, for 18°. Carried out with the small saturator electrode. Series A.

Concentration	Temp.	mm. Hg CO_2	Vols. % CO_2 combined	p_H	$p_{K'_1}$ 18°	
0·85 % NaCl	19·0	49·3	35·8	7·03	6·26	$Y=6$
70 cc. $NaHCO_3$ ($T=43·0$)	19·0	156·9	45·9	6·63	6·24	Phosphate
6 cc. KH_2PO_4 (0·066m)	19·0	48·2	34·8	6·45	6·27	
24 cc. Na_2HPO_4 (0·066m)	19·0	16·3	28·9	6·41	6·24	
0·02m phosphate	19·0	38·3	33·6	6·44	6·25	
0·145n $Na^\cdot$				Mean	6·25	
0·004n $K^\cdot$						
0·42 % NaCl	18·0	51·5	38·5	7·06	6·28	$Y=5$
70 cc. $NaHCO_3$ ($T=43·0$)	18·5	28·9	33·4	7·25	6·28	Phosphate
6 cc. KH_2PO_4 (0·066m)	18·5	53·6	37·5	7·05	6·29	
24 cc. Na_2HPO_4 (0·066m·	19·0	156·9	48·2	6·66	6·25	
0·02m phosphate				Mean	6·28	
0·0718n Cl'						
0·101n $Na^\cdot$						
0·004n $K^\cdot$						

Table XLVIII. Determinations of $p_{K'_1}$ for 18° in cane sugar solutions (0·501m) with sodium bicarbonate (*circ.* m/100). Carried out with E VI and E III.

Concentration	Temp.	mm. Hg CO_2	p_H	$p_{K'_1}$	Vols. % CO_2 combined	
0·501m saccharose	18·0	519·9	6·03	6·36	27·6	$Y=16·5$
$T=27·7$	18·0	117·3	6·66	6·38	28·1	E VI
0·0125n HCO'_3	18·5	28·6	7·29	6·39	27·6	Series A
0·0125n $Na^\cdot$	19·0	16·9	7·47	6·34	27·6	
	19·5	523·4	6·03	6·40	27·7	
	18·5	34·7	—	(6·31)	27·9	
				Mean 6·38	Mean 27·7	
0·501m saccharose	20·5	43·9	7·098	6·471	—	$Y=16·5$
$T=22·44$	20·5	43·9	7·092	6·465	—	E VI and E III
0·01008n HCO'_3	20·5	41·4	7·102	6·450	—	Series B
0·01008n $Na^\cdot$	20·5	41·4	7·102	6·450	—	
	20·5	43·6	7·095	6·465	—	
	20·5	43·6	7·095	6·465		
				Mean 6·461		

CHAPTER IX

PRELIMINARY CONSIDERATIONS CONCERNING THE MODE OF COMBINATION OF CARBONIC ACID IN THE BLOOD

In the first seven chapters of this work the relations existing between the apparent hydrogen ion activity, the CO_2 tension and the combined carbonic acid of the blood have been developed. These relations depended on the assumption that all the combined carbonic acid in the various phases of the blood is present as bicarbonate[1], but on further scrutiny it will be obvious this proviso is by no means proved by the fact that the apparent hydrogen ion activity can be calculated from the modified Henderson-Hasselbalch equations. On the contrary it will be perceived that in the empirically determined "constants," corrections for combined carbonic acid not in the form of bicarbonate can very easily be hidden.

When I started these investigations I thought that the corroboration of Henderson's and Hasselbalch's "proof" that all the combined carbonic acid in the blood was present as bicarbonate, would not involve great theoretical difficulties so that by taking the heterogeneity of the solution into consideration it would be feasible to determine whether the evidence was really sound. But the more I have pondered over the theoretical conditions, the greater the difficulties have seemed to be. I have nevertheless, as far as I have been able, fulfilled my original plan, inasmuch as, it seems to me, questions of considerable interest have arisen but I have furthermore endeavoured to establish the proof in another way in order to avoid the most important difficulties. The evidence will be found in chapter XI but for the present it will be shown how far we can go with the original idea. The value that gives the greatest trouble is the apparent activity coefficient of the bicarbonate ion in the liquid phase of the blood corpuscles, and uncertainty in evaluating this will produce a significant error in the calculations in this chapter and chapter XII. I do not think however the conclusions arrived at can be disputed from a qualitative point of view but I quite realise that quantitatively, especially in this chapter, they may rather diverge from the results that may eventually be obtained.

In chapter III an equation was evolved for the calculation of the apparent hydrogen ion activity in the water phase of a two-phase system. With this equation (115) we can estimate the amount of bicarbonate which will be found in the serum proteins and haemoglobin if the combined carbonic acid is present as bicarbonate:

$$(115) \quad a_{H'} = \lambda_{(1)} \frac{P_{CO_2}\alpha}{7{\cdot}60\,\beta},$$

[1] No account is taken here or henceforth of the small quantities of monocarbonate found in the blood (cf. chapter II).

the condition for which is

$$(117) \quad \lambda_{(1)} = K'_1 \frac{100 - q(1-d)}{100},$$

from which we obtain $$d = \frac{100\,(\lambda_{(1)} - K')}{K'_1 q} + 1, \quad \text{.....(151)}$$

in which the significance of the constants is discussed in chapters II and III. Let us first consider serum. We found in chapter V

$$\lambda_{(1)} = \lambda_{(s)} = 10^{-6\cdot294} = 5\cdot20 \times 10^{-7}.$$

K'_1 can be determined from the equation

$$(143) \quad -\log F_a\,(HCO'_3) = 0\cdot46\,\sqrt[3]{c}.$$

For the determination of the apparent activity coefficient we only lack the value of c.

Numerous and thorough investigations by Hamburger [1902], Hedin [1915], Ege [1920] and many others have shown that serum is almost isotonic (isosmotic) with a 0·9 % sodium chloride solution. For further information respecting this I would refer the reader to Rich. Ege's thesis. It would be erroneous however immediately to conclude that c was the same as in 0·9 % NaCl solution, that is $0\cdot154n$, because NaCl mols are not the only osmotically active ones. Now the mols which are not electrolytes are only present in such a small concentration in serum that for this purpose we can neglect them entirely. But at the reactions here in question there are also some protein anions present in serum which neutralise corresponding amounts of cations. The quantity of these never exceeds $0\cdot02n$ and presumably is usually about $0\cdot01n$, so we are justified in estimating c at between $0\cdot16$ and $0\cdot18n$.

The volume of the disperse phase in serum is certainly very nearly 8 % and putting this in equation (151) we obtain

$$c = 0\cdot16 \quad K'_1 = 10^{-6\cdot260} = 5\cdot50 \times 10^{-7} \quad d = 0\cdot32,$$
$$c = 0\cdot18 \quad K'_1 = 10^{-6\cdot251} = 5\cdot61 \times 10^{-7} \quad d = 0\cdot07.$$

The calculation is rather uncertain because a slight error in K'_1 or in $\lambda_{(s)}$ has a great effect, but it seems as though some bicarbonate is dissolved in the serum proteins.

Table XLIX. Calculation of the value of d in the blood corpuscles with equation (151).

		d	
c	$K'_1 \times 10^{-7}$	$q=40$	$q=35$
0·20	5·30	0·51	0·44
0·225	5·41	0·47	0·40
0·250	5·52	0·42	0·35

The concentration of cations in the water phase of the blood corpuscles is not certainly known. It has been estimated here as between 0·2 and 0·25. The reason for this will be discussed later in chapter XII.

$\lambda_{(1)} = \lambda_{(c)}$ in horse blood cell fluid, according to the measurements reported in chapter VII, is about 6·37. The volume of the disperse phase in blood cells

according to Ege's determinations is between 35 % and 40 %. If d is calculated with these values we obtain the results given in Table XLIX, using equation (58) with the constant 0·40, as a large part of the cations of blood cells consists of potassium.

From the table it will be seen that the value of d, under the given assumptions, lies between 0·35 and 0·51.

As emphasised above it is quite possible that the calculation may be rather uncertain quantitatively but there is hardly any doubt that d is really a positive quantity. If $d = 0$ and q and $\lambda_{(c)}$ at the same time have the values employed in the calculation above, we can estimate K'_1 and c since in that case the following holds:

$$K'_1 = \frac{100}{100 - q}\lambda_{(c)},$$

and therefore for $q = 40$ we get $K'_1 = 7{\cdot}12 \times 10^{-7}$ and $c = 0{\cdot}74n$, and for $q = 35$, $K'_1 = 6{\cdot}57 \times 10^{-7}$ and $c = 0{\cdot}55n$. These values for c are undoubtedly too high.

It may therefore be taken as settled that a part of the bicarbonate is dissolved in the haemoglobin provided that all the combined carbonic acid of the blood is really present as bicarbonate, and the calculations also show that the bicarbonate is about half as soluble in haemoglobin as in a roughly $n/4$ KCl solution (the dispersion medium).

This result is by no means antagonistic to what we already know about the solubility of salts in proteins. Thus S. P. L. Sörensen [1915–1917] found that ammonium sulphate was freely soluble in egg albumin but, on the other hand, it compels us to inquire whether a part of the combined carbonic acid is not present in some other form than the bicarbonate ion (or undissociated bicarbonate in the haemoglobin phase). It would be interesting to estimate first what amount of the combined carbonic acid of the blood may be expected to be dissolved in the protein phases according to the above calculations.

As an example let us take horse blood at 18° with a $p_{H'(s)}$ value of 7·40 and at a CO_2 tension of 40 mm., and further we will assume the volume of the blood cells is 40 % of the total volume of blood, which according to Fig. 10 corresponds to 19·6 vols. % combined O_2 (Haldane 106). We now calculate with (130) the combined carbonic acid (B) making the necessary correction with the help of Fig. 10, and we obtain 58·4 vols. %. From Fig. 7 we read that the partition ratio of combined CO_2 at this reaction (D) is 0·66.

In 100 cc. blood therefore there are 58·4 cc. combined CO_2 (0° and 760 mm.), of which 40·6 cc. are present in the 60 cc. serum, equivalent to a content of 67·6 cc. in 100 cc. serum; and in 40 cc. blood cells there are consequently 17·8 cc. combined CO_2 corresponding to 44·6 cc. in 100 cc. blood cells. Of the 60 cc. serum the water phase is 55·2 cc. and the protein phase (8 %) 4·8 cc., and if we assume d is 0·35, we obtain 39·4 in the water phase corresponding to 71·3 cc. in 100 cc. and 1·2 cc. in the protein phase which is equivalent to 25·0 cc. in 100 cc. protein. If we carry out a similar calculation for the blood corpuscles and put $q = 40$ and $d = 0{\cdot}45$, the water phase becomes 24 cc. and

the protein phase 16 cc. There are then 13·7 cc. in the water phase, equivalent to 57·2 cc. in 100 cc. and 4·1 cc. in the protein phase, equivalent to 25·7 cc. in 100 cc. In the two disperse phases there are altogether 4·1 + 1·2 = 5·3 cc. combined CO_2 which is equivalent to about 9 % of the total quantity of combined CO_2. We have found that 71·3 vols. % combined CO_2 are present in the water phase of serum, while there are only 57·2 vols. % in the water phase of the blood cells. Now the apparent activity coefficient of the bicarbonate ion is very nearly identical in the water phase of serum and blood cells because the greater cation concentration in the blood cells is almost balanced by the smaller Milner effect of the potassium ions when it is compared with the sodium ions, and the relation between the amounts of combined CO_2 in the two water phases will thus directly afford a measure of the relation between the activity of the bicarbonate ion in them, so that

$$\frac{a_{HCO_3} \text{ in the water phase of the blood cells}}{a_{HCO_3} \text{ in the water phase of the serum}} = 0{\cdot}8.$$

This result is by no means at variance with what we should expect, as it will be shown in chapter XII that on the basis of Ostwald's [1890] and Donnan's [1911] partition law such a distribution is to be looked for.

The above results are condensed in Table L.

Table L.

	Vols. % combined CO_2	Cc. combined CO_2
100 cc. blood at p_H· 7·40 and 40 mm.	58·4	58·4
In the serum of the above (60 cc.)	67·6	40·6
„ water phase of the serum (55·2 cc.) ...	71·3	39·4
„ protein phase of the serum (4·8 cc.) ...	25·0	1·2
„ blood cells (40 cc.)	44·6	17·8
„ water phase of the blood cells (24 cc.) ...	57·2	13·7
„ protein phase of the blood cells (16 cc.)	25·7	4·1

The amount of CO_2 combined in a form other than bicarbonate would thus appear to be small and, according to the above calculations, at most 9 % of the total. We will now try to get an idea of what form of combination this might theoretically be.

The combined carbonic acid is determined as the difference between the total dissociable combined CO_2 and the dissolved amount calculated by Henry's law. The relative absorption coefficient of blood and serum has been determined indirectly by Bohr [1905], the assumption being that it is the same for all gases. This is of course only approximately true but the divergence is not so large that a grave error can arise if the CO_2 tension is not too high. It is however possible that CO_2 could be adsorbed at the interface between protein and the dispersion medium. That such an adsorption actually takes place in blood is claimed by Findlay for various gases as will be referred to in the next chapter but he undoubtedly overestimates the significance of his experiments. It must also be noted that $\lambda_{(c)}$ could not be independent of the reaction if such an adsorption took place for reasons identical with those that will be advanced at the end of this chapter against the existence of a protein-carbamino-acid.

The possibility may also be raised that some of the bicarbonate may be present as undissociated protein bicarbonate (complex salt). This assumption is however not quite correct because in alkaline reactions only very small or negligible amounts of protein cations are present, since proteins function almost entirely as acids and so there can be no question here of undissociated protein bicarbonate. At reactions in the region of, or more acid than, $10^{-7 \cdot 0}$ some haemoglobin cations are in all probability formed so that there is the possibility of the existence of undissociated haemoglobin bicarbonate and in such a case $p\lambda_{(c)}$ might decrease with increasing hydrogen ion activity, but in chapter VII we saw this was not so. We can therefore conclude that undissociated bicarbonate does not exist in sufficient quantity to compromise our theories and calculations. Further it is possible that the bicarbonate ion might be adsorbed at the interface between protein and salt solution. As a matter of experience however such an adsorption only takes place when the disperse phase has a charge of opposite sign to that of the adsorbed ions, and so the theory of the adsorption of the bicarbonate ion by proteins is untenable for the same reason as applies to undissociated protein bicarbonate. Furthermore the problem of the adsorption of the ions is presumably identical with the problem of the large Milner effect of the colloid ions, according to the views of which Prof. Bjerrum has kindly given me the benefit. There remains the possibility that some of the CO_2 combines with the protein in some other form than protein bicarbonate. Such a form of combination has been stated by Siegfried to be protein carbamino-acid.

It will be clear to the reader that the combined carbonic acid referred to in this work is only the dissociable combined carbonic acid, that is to say the carbonic acid which can be liberated by the addition of acid or by diminishing the CO_2 tension; that which can only be split off from the organic molecules by such vigorous processes that the latter are destroyed is naturally not included, it can moreover be shown that this dissociation of the carbonic acid combination of the blood is completely reversible. If protein carbamino-acid took part in the reversible carbonic acid combination $\lambda_{(c)}$ (or $\lambda_{(m)}$) could not be constant in the fluid of the blood cells because the non-ionised combined H_2CO_3 would then vary with the CO_2 tension. This evidence that all the combined carbonic acid is really ionised in the blood is well supported by the determinations of $p\lambda_{(m)}$ in chapter VII, but is weakened to some extent by the determinations being so extremely difficult to carry out. But it also involves the assumption that q and d in equation (117) do not vary appreciably with the hydrogen ion activity. From a close examination of (117) however it appears the variations must be fairly large to make themselves felt in the determinations in question.

As it would certainly involve very great experimental difficulties to proceed further with the questions raised in this chapter there is every inducement to examine the literature for experiments which can throw light on these problems.

In conclusion it may be remarked that the experiments and views put forward give no information as to whether the bicarbonate dissolved in the protein phase is present exclusively as ions, or as undissociated salt as well.

RÉSUMÉ.

It has been shown that a small part of the combined carbonic acid is not present in the dispersion medium of the serum and blood cells, and the manner of combination of this amount of combined acid is discussed. It is probable that it is dissolved in the protein phases.

CHAPTER X

A BRIEF HISTORICAL REVIEW OF THE OLDER THEORIES OF THE COMBINATION OF CO_2 IN THE BLOOD.

The first to extract gas out of blood was John Mayow of Oxford (quoted from P. Bert), who reported his results in a paper in 1674. He thought the gas obtained was what we now call oxygen.

In 1783 Pietro Moscati of Milan stated he had extracted a gas out of blood and he believed he had proved it to be carbonic acid (fixed air), but his evidence was not good as by allowing blood to stand for 24 hours with lime water he obtained a precipitate [1784].

In 1799 Humphrey Davy proved that CO_2 could be driven out of blood by heating it, and in 1814 Vogel showed that CO_2 could be pumped out of blood. Up to 1837 when Magnus finally established that CO_2 could be pumped out of blood, the question was one of considerable interest as it was closely bound up with the current discussion on where oxidation took place in the organism.

Lavoisier had originally discussed whether oxidation occurred in the peripheral circulation or in the lungs. In his later works he claimed to have proved it took place in the lungs (according to W. Edwards and Ch. J. B. Williams). He believed that the carbon in a state of fine division was carried by the blood to the lungs and burned up there in association with oxygen. It became therefore of prime importance at that time to ascertain whether carbonic acid (CO_2) was free in the blood or only in a state of combination. In those days it was evidently not considered sufficient that carbonic acid could be driven out of the blood by heat, or by treatment with another gas such as hydrogen or atmospheric air, because it was supposed these gases replaced carbonic acid in loose combinations although it was partly realised similar processes took place in the lungs.

The first to have pumped CO_2 out of blood seems to have been, as already mentioned, Vogel [1814] and, apparently almost simultaneously, Vaquelin. This last investigator's experiments were never published by himself but were recorded by W. Edwards [1824].

Between 1814 and 1837, Scudamore, Brande and others succeeded in pumping CO_2 out of blood, while Darwin[1], Davy [1803], Stromeyer [1832], Misterlich, Gmelin and Tiedemann [1834] failed in an attempt to do so.

Stevens' [1832, 1835] experiments and theories are especially interesting. He was a Scotsman who lived the greater part of his life in the then Danish West Indies, and he seems to be almost forgotten in scientific literature, which is partly due to the immense range of his works, but he appears to have introduced a treatment for cholera which although based on faulty experimental material, constituted a real step forward at the time (cf. *Observations on the Nature and the Treatment of the Asiatic Cholera*, London, 1853).

In his book of 1832 Stevens put forward the theory, on pp. 22–26, that the carbonic acid of the blood is present as alkaline carbonates and as free CO_2, comparing the change of reaction of serum on exposure to the air with the change which takes place in the alkaline mineral water highly charged with CO_2 from a spring at Saratoga in New York State, when it is allowed to stand in the air.

In 1835 he communicated a series of experiments to the *Philosophical Transactions* which showed that he was unable to pump CO_2 out of venous blood immediately after it was taken but he succeeded after the blood had stood for some time in contact with hydrogen or air. It had been repeatedly shown that blood could absorb considerable amounts of CO_2—a fact which Stevens himself demonstrated experimentally—but he could not get the CO_2 out again without letting the blood stand some time in the air or hydrogen. Stevens thought therefore that these gases liberated CO_2 allowing it to be pumped out. It is perfectly clear to us now that the disagreement between the different observers was due to their gas pumps being of different degrees of efficiency and those who had the worst pumps could not pump CO_2 out of freshly drawn blood. The one who finally proved with certainty that the blood contained CO_2 which could be expelled by blowing hydrogen through it or by the exhaust pump was Magnus. His article in Poggendorff's *Annalen* of 1837 is still worth reading not only on account of its historical interest but also in view of the great insight of the author, and on perusing it, it is striking, as is the case with so many other works of the first half of last century, how little physiological problems have changed their character and how often the same phenomena are rediscovered.

A sentence in an article in 1834 by R. Hermann of Moscow is of interest.

On the 5th September, 1833, Drs Stevens, Markus, Wyllie, Jänchen and R. Hermann met in Moscow for an investigation.—"Nachdem man sich einstimmig darüber ausgesprochen hatte, dass das Serum als eine Flüssigkeit

[1] Quoted from Williams [1835].

zu betrachten sei, die neben doppelt-kohlensaurem Natron noch freie Kohlensäure erhalte (on account of the reaction of the serum), ging man zu Untersuchung des Blutes über."

It was not all however who realised that blood contained free carbonic acid. Misterlich, Gmelin and Tiedemann [1834], and also Davy [1803] and others, thought that blood only contained combined CO_2 while one gets the impression that Magnus [1837] regarded all the CO_2 as free. Even in the middle of the sixties W. Preyer [1867] could proclaim with success that no free CO_2 was to be found in the blood because it could not be present in an "alkaline" liquid. When Zuntz [1868] pointed out that free CO_2 might be found in blood when it was in equilibrium with an atmosphere containing CO_2 the question was finally decided. P. Bert however in a paper in 1878 opposed this view.

Magnus, whose technique was a considerable advance on that of earlier workers, contributed analyses which even in those early days gave fairly correctly the quantity of CO_2 in the blood. These experiments were the best until 1857 when Fernet in France and Lothar Meyer in Germany published extensive investigations on the subject. Fernet's technique—which Meyer also used—consisted in driving the gas out of blood by means of a vacuum produced with the help of water vapour and afterwards analysing the gas driven out. Fernet and Meyer thought the combined CO_2 in blood could be divided into two kinds, namely that which could be liberated with a vacuum and heat alone, and that which required the addition of acid. About this time Bunsen's first absorption coefficients appeared and it was then possible to calculate the amount of combined CO_2 in the blood. The values found by Fernet and Meyer were however too low—in fact in the case of CO_2 they were no better than those of Magnus—on account of the relatively bad vacuum. Fernet proposed the theory that the CO_2 of the blood was combined with phosphates as a double salt but Heidenhain and Meyer [1863] showed a few years later that Fernet's analyses with respect to the double salt were not correct, and Sertoli proved in 1868 that the amount of free phosphates in the blood was so small that it could be entirely neglected as regards its capacity for combining with CO_2 in view of the relatively large amount of combined CO_2 which had been found. Later investigations have fully supported Sertoli's findings and according to the latest experiments which have been collected amongst others by Poul Iversen [1920], blood and serum contain about $m/1000$ dissociated phosphate. The combination of CO_2 in phosphate solutions has nevertheless had no small influence on our knowledge of CO_2 compounds in blood, as it is a rather simple example of a CO_2 combination in an electrolyte the dissociation of which varies with the reaction. Heidenhain and Meyer [1863], Setschenow [1879], Henderson and Black [1908] and K. A. Hasselbalch [1910] have all used phosphates as a solution comparable with blood, and the author will also report some similar experiments later on in this work.

Ludwig [1865] and his collaborators Schöffer [1861, 1868] and Setschenow [1859], in the latter half of the "fifties" and in the "sixties," carried out a

number of experiments upon the CO_2 compounds in blood, and these investigators also considered that all the CO_2 could not be extracted out of the blood (with Ludwig's mercury air-pump) without the addition of acid. It was not until Pflüger [1864] in conjunction with Geissler constructed a new pump that the extraction was so complete that the yield was not increased by adding acid. Pflüger also showed that all the CO_2 could not be pumped out of serum (when it was separated from the blood corpuscles) without adding acid. Pflüger finally showed that in addition to the CO_2 which blood yielded to a vacuum a further supply could be liberated from sodium carbonate. Pflüger fully realised that pumping out in the presence of sodium carbonate was accompanied by a transformation of monocarbonate into bicarbonate and it was owing to this that the CO_2 was driven out (cf. H. Rose [1835] and Marchand [1846]). He considered the acids which drove out the CO_2 were haemoglobin and the serum proteins. Hoppe-Seyler thought that haemoglobin only acted as an acid under the influence of the exhaust pump, being destroyed during the pumping, in spite of the fact that such an assumption is unnecessary for explaining the phenomenon, and, so far as I am aware, a proof has never been furnished; the hypothetical acid could only drive out a very small quantity of CO_2, because Gaule [1878] and Zuntz [1882] have shown that after pumping out, blood combines with almost as much acid as before.

Pflüger [1864] thought the difference between blood and serum consisted in the fact that less acid was formed in serum on pumping out than in the blood corpuscles.

Sertoli [1868] and Setschenow [1879] both showed that globulin is an acid which can expel CO_2 from sodium carbonate, and taking everything into consideration there is now hardly any doubt that the proteins really possess an acid character. As it is also an accepted fact that they can combine with acid their ampholytic nature is proved. It is an observation over 100 years old that the proteins function both as bases and acids. About the beginning of the present century they were classified as ampholytes, at which time the peculiar properties of this group of electrolytes became known, and their physico-chemical characteristics were thoroughly worked out by Bredig, Walker, Hardy and Pauli, and later particularly by Sörensen and Michaelis and their numerous collaborators, while Henderson and Spiro and Hasselbalch have rather elaborately dealt with the consequences of the ampholytic character in relation to CO_2 combinations in the blood.

In 1882 Zuntz, in Hermann's handbook, collected the investigations relating to the combination of carbonic acid in the blood in a deservedly celebrated monograph. He was able, largely on the basis of his own and Setschenow's experiments, to enunciate the theory for the combination of carbonic acid, that CO_2 was combined with the alkali of the blood in such a way that carbonic acid and the blood proteins divided the alkali between them, and also the haemoglobin at high CO_2 tensions acted as a base and thus combined with CO_2 itself.

Some years before, Setschenow [1879] had come to a very similar conclusion about the combination of CO_2, the only difference being that he interpreted CO_2 combinations at high CO_2 tensions not as a production of haemoglobin bicarbonate but as a more specific reaction for haemoglobin.

The view which is held to-day regarding the CO_2 compounds in the blood only slightly differs from Zuntz's, the most important advance being the knowledge that the proteins belong to the group of ampholytes. The premises upon which Zuntz built his theory were pre-eminently the following:

(1) The well established fact that proteins combine with both acids and bases.

(2) Pflüger's observation that haemoglobin can drive CO_2 out of sodium carbonate, in conjunction with Sertoli's and Setschenow's demonstration of the same thing for *globulin* and *paraglobulin*.

(3) Setschenow's demonstration that the blood can combine with more CO_2 than corresponds to the difference between the cations and anions the dissociation of which varies with the reaction (expressed in modern terminology).

Of these premises only Pflüger's has later become open to doubt as Buckmaster tried to show in 1917, but the latter author is certainly wrong. His results can only be explained by his having added too much alkali so that the amount of bicarbonate formed is so small in relation to the amount of monocarbonate that there is not a measurable CO_2 tension in the system and thus CO_2 could not be pumped out. Quite recently Adolph [1920] has also shown Buckmaster was in error, but the latter's experiments have raised so much discussion that I think it worth while reporting one of two experiments I have undertaken which were in good agreement and which well support Pflüger's experiments.

Experiment.

Defibrinated ox blood kept in the ice cupboard from 19. viii. till the beginning of the experiment.

The blood corpuscles were separated by centrifuging.

On 20. viii. 6·98 cc. of the blood corpuscle emulsion in equilibrium with 40·5 mm. CO_2 were pumped out into an acid-free evacuation receiver. It contained 39·1 vols. % CO_2 and 38·2 vols. % O_2 (0° and 760 mm.).

On 21. viii. 7·12 cc. blood cell emulsion in equilibrium with 40·9 mm. CO_2 and 146·1 mm. O_2 at 18° contained 38·5 vols. % CO_2 and 32·3 vols. % O_2 on pumping out in the presence of boric acid.

In an acid-free evacuation receiver were put 6·64 cc. NaOH in equilibrium with 40·9 mm. CO_2. This NaOH, according to previous estimations, combined with 219·7 vols. %, and there was physically absorbed 5·01 vols. %, that is altogether 224·7 vols. % (0° and 760 mm.).

Further there was put in the receiver 10·55 cc. of the blood cell emulsion.

	0° 760 mm.
In 6·64 cc. $NaHCO_3$	14·92 cc. CO_2
Therefore 10·55 cc. of the above blood cell emulsion in equilibrium with 40·9 mm. CO_2 and 146·1 mm. O_2 contained	4·06 „ „
	18·98 „ „

	0° 760 mm.
In one hour there was pumped out	18·35 cc. CO_2
After the addition of boric acid for 7 mins. a further	0·65 „ „
Each pumping out took place at 38°.	19·00 „ „

Pflüger's [1864] phenomenon that all the combined CO_2 of the blood can be pumped out and further CO_2 can be driven out by addition of sodium carbonate shows beyond doubt that substances are present in the blood which act as acids at reactions more alkaline than the neutral point, but does not prove that all the combined CO_2 in the blood is in the form of bicarbonate because every compound of CO_2 with another substance will be dissociated as soon as the CO_2 tension is diminished.

It is justifiable to assume that the electrolytes of the blood the dissociation of which varies with the reaction are mainly proteins, particularly paraglobulin (Setschenow [1879]) and haemoglobin (Zuntz [1868], Mathieux and Urbain, Setschenow [1879], Bohr [1891, 1898, 1904, 1909], Hasselbalch [1916, 2], Campbell and Poulton [1920] and many others). But Setschenow [1879] has shown that *lecithin* also acts as a weak acid, and it is known that phosphates act in the same way although as already mentioned they are only found in quite small concentrations.

There are some theories in the literature which differ from the Zuntz-Setschenow theory. The first is Bohr's [1904]. He believed the combination of CO_2 took place in the following way:

$$CO_2 + A_B\text{Hb}A_s \rightleftarrows CO_2\,(A_B\text{Hb}) + A_s$$

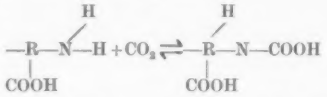
(the symbols being Bohr's own). In other words he imagined haemoglobin contained an acidic and a basic group and that at the same moment in which it combined with CO_2 it split off a new acid. Bohr has worked out this relation on the assumption that there is no alkali in the system and that the process is independent of the reaction of the solution.

So far as I can see the only proof which Bohr has tried to give that the process really proceeds is that the CO_2 compounds in a haemoglobin solution and in the blood can be calculated by an equation derived under the above assumptions, but it is generally accepted a proof of this kind is always very weak. Although I cannot assert that a reaction of the kind in question does not take place particularly when developed according to Siegfried's [1905, 1, 2; 1908, 1, 2; 1910] theory, as will be explained later on, it is probable at any rate that such a process only proceeds to a very slight extent. Bohr has reported numerous experiments on CO_2 compounds in pure haemoglobin and claims to have shown that various modifications of haemoglobin exist which can pass into one another but he is unable to show how. This complicates Bohr's view still more and it seems to me it would be useless to express an opinion on the CO_2 compounds in pure haemoglobin solutions without carrying out fresh experiments.

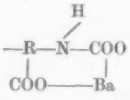
Siegfried and his collaborators have shown that by treating amino-acids

with CO_2 in the strongly alkaline barium hydroxide or calcium hydroxide solution at a temperature about freezing point a complex compound of carbonic acid and amino-acids is formed and he has further shown that the same compound is also formed even if no Ba or Ca is present in the solution. When a solution in which a complex CO_2-amino-acid compound is formed is heated and Ba or Ca is present, a precipitate of the carbonates of these elements will separate out.

Siegfried believes that complex compounds of the amino group and CO_2 are formed according to the following scheme:



and that they are strong acids which form salts as follows:



(Siegfried seems to imply by this formula that the barium salt is not dissociated; this is however unimportant in the present connection.)

In my opinion Siegfried has only shown that CO_2 is combined in a complex manner with the amino group in the cold and in a very alkaline reaction, and he appears to have failed to supply any proof that the compounds formed are acids, let alone strong acids.

Siegfried [1908, 2] claims to have shown that proteins form with CO_2 complex compounds similar to the amino-acids. The experiments he considers most telling in this respect are certainly not convincing and they are probably quite wrongly interpreted. Siegfried passes CO_2 through a protein solution and shows that the conductivity increases more than can be accounted for by the CO_2 alone. He concludes from this that dissociated carbamino-acids are formed but it seems to me more natural to ascribe the effect to the formation of dissociated protein bicarbonates. Siegfried has *assumed* that protein carbamino-acids are formed at the temperature and reaction of the body and that it is a reversible reaction and they would be formed in greater or less amounts according to the prevailing CO_2 tension, and further that these compounds participate in the transport of CO_2 from the tissues to the lungs. This view has gained some acceptance (cf. for example Hammarsten [1914] and Wilstätter and Stoll [1918]) but in my opinion there is no evidence that the compounds exist or are dissociable at physiological temperatures, reactions and CO_2 tensions. *Per contra* it has naturally not been proved such bodies do not exist.

The third theory relating to CO_2 compounds in the blood is mainly supported by experiments of Findlay and his collaborators [1908, 1910, 1911, 1912, 1913, 1915]. The theory itself has not been actually propounded but Findlay's

experiments and his whole method of reasoning in his papers certainly suggest the enunciation of such a theory. Findlay's idea is that gases can be adsorbed by colloids and that it is possible such an adsorption takes place in the blood. The experiments of Findlay and his collaborators have from a physiological standpoint had an unfortunate fate. In 1908 Findlay formulated the working hypothesis referred to and investigated in the following years the adsorption of various gases in several colloid solutions. By this great pioneer work Findlay has brought to light a considerable number of previously unknown conditions which still await further investigation. From his experiments he could conclude that inactive gases are frequently adsorbed in colloid solutions, and that in some cases CO_2 is adsorbed and in others combined (as bicarbonate).

In 1911 Findlay and Creighton reported some experiments on the absorption of oxygen, nitrogen, nitrous oxide, carbon monoxide, and carbon dioxide in blood and serum but these experiments, from which he concluded that oxygen, nitrogen, nitrous oxide and carbon monoxide were adsorbed by the proteins, are from a technical point of view so indifferent that the results are far from convincing. Findlay and Creighton have not taken sufficient notice of the fact that there are large quantities of combined O_2 and CO_2 in the blood which are dissociable and that it is necessary to make sure the compounds of these gases have not changed if one wishes to determine the absorption conditions of another gas by Findlay's technique; but this has certainly not been the case in the experiments referred to. In physiological circles this has been generally known since the days of Fernet and Meyer, and it is only the fact that Findlay has been trained as a chemist that makes it intelligible he should overlook the point.

I will quite briefly show from a single series that Findlay and Creighton's experiments are much too uncertain to allow of any conclusion being drawn. Findlay and Creighton have determined the absorption coefficient (Ostwald's) at a number of different tensions. It is easy with the help of the equation first employed by Fernet [1857] and Meyer [1857] to estimate the absorption coefficient between two tensions. I have performed this and then calculated this value as the relative absorption coefficient at the temperature of the experiment, in pure water.

A few of the experiments will be found recalculated in the following table:

CO in blood which had been exhausted before the saturation[1]		CO in blood not exhausted	
mm. Hg CO_2	Relative absorption coefficient	mm. Hg CO_2	Relative absorption coefficient
751–871	0·32	751–944	1·12
871–1056	1·61	944–1144	1·52
1056–1140	1·08	1144–1371	0·95
1140–1274	1·07	1371–1434	2·75
1274–1543	0·90	1434–1528	1·80

It appears further from other experiments that the absolute values found for the absorption coefficients are on an average much lower than they have

[1] With a water exhaust pump.

been found before, in fact inexplicably low. There is no alternative but to conclude that Findlay's experiments with blood are subject to such a large technical error that they cannot be utilised. The issue itself which Findlay has raised is very interesting. Jolyet and Sigalas [1892] claim to have shown that blood absorbs more nitrogen than it should if the absorption coefficient were the same as for pure water. Bohr [1909] and later Buckmaster have been able to confirm this although Buckmaster [1911–1912] only finds about 10% too much. There is yet another fact which might be brought forward in support of the contention. Bohr [1905] has found the relative absorption coefficient for hydrogen in blood is 0·92 and Fahr [1912] found approximately the same values.

In serum Bohr estimated 0·975 as the coefficient for hydrogen.

In serum Fahr estimated 0·94 to 0·95 as the coefficient for hydrogen.

In blood cells Bohr estimated 0·81 as the coefficient for hydrogen.

In blood cells Fahr estimated 0·78 to 0·88 as the coefficient for hydrogen.

R. Siebeck [1909] has found values for nitrous oxide in blood, blood cell solutions and haemoglobin solutions which are a little over 1 (for blood 1·03), while Lindhard [1914] found about 0·97 for blood; and for serum Siebeck found the same value with nitrous oxide as Bohr found with the hydrogen[1].

The disagreement between the depression of solubility for hydrogen and nitrous oxide is pronounced. Should this be supported by experiments it points strongly to complicating factors in the solubility of the gases in protein solutions.

Thorup [1887] states he has demonstrated the existence of a CO_2-haemoglobin compound spectroscopically. He has shown that reduced haemoglobin when treated with high tensions of CO_2 develops a different absorption spectrum. From a modern physico-chemical point of view we cannot judge by this method whether complex combinations are formed or whether the haemoglobin becomes ionised, because electrolytes with characteristic spectra usually change their absorption bands on ionisation.

In conclusion I will discuss a view put forward by Jaquet [1892]. He has asserted that the CO_2 combination curve of the blood, when plotted with the CO_2 tensions along one axis and the combined CO_2 along the other, is shifted parallel with itself on addition of acid or base to the blood. If Jaquet's statement were right it would prove that the CO_2 combination with haemoglobin was independent of the reaction and thus was not a bicarbonate compound as it appears to be from the reasoning in the next chapter. But Jaquet's experiments are a long way from proving his contention because in the first place the curves are not truly parallel and secondly they are produced by points corresponding to experiments with different samples of blood.

[1] D. D. van Slyke and W. C. Stadie (*J. Biol. Chem.* 1921, **54**, 1) in accordance with earlier investigators have found greater quantities of N_2 in the blood than is to be expected from Henry's law. A similar observation was made by Smith, Dawson and Cohen (*Proc. Soc. Exp. Biol. and Med.* 1919–20, **17**, 211), quoted from van Slyke and Stadie. At the Finsen Institute I have myself frequently had a similar experience.

RÉSUMÉ.

A search of the old literature does not disclose any facts which would lead us to abandon Zuntz's theory modified in accordance with modern physico-chemical requirements.

CHAPTER XI

FURTHER CONSIDERATIONS AND EXPERIMENTS ON THE NATURE OF THE COMBINATION OF CO_2 IN THE BLOOD, ILLUSTRATED BY THE "CARBONIC ACID COMBINATION" CURVE[1].

In a solution in equilibrium with air containing CO_2, a quantity of CO_2 is found to be dissolved according to Henry's law, as repeatedly stated. This CO_2 can be extracted from the solution by diminishing the CO_2 tension in the gas phase over the solution, and further by this process more CO_2 which must have been reversibly "combined" in the solution can often be liberated.

It has frequently been stated in earlier chapters that the combined CO_2 may be present

(1) as adsorbed CO_2 if the solution is heterogeneous,

(2) as carbonate ions (monocarbonate and bicarbonate),

(3) as undissociated carbonate (complex salt),

(4) as adsorbed carbonate which will presumably be identical with (3), since the molecular groups lying on the surface in a high molecular solute may be expected under certain circumstances to form compounds with the carbonate,

(5) as a dissociable combination in which CO_2 takes part after assumption into the molecule, *e.g.* carbamino-acids.

At reactions more acid than $a_{H^{\cdot}} = 10^{-8}$ we can regard the carbonate ions present solely as bicarbonate (cf. chapter II).

We will first examine systems in which adsorption combinations and complex salts are not found. In general, the following equation for the equilibrium of the positive and negative charges is valid for any solution whatever:

$$C_{M^{\cdot}{}_{\mathrm{I}}} + 2C_{M^{\cdot\cdot}{}_{\mathrm{II}}} + 3C_{M^{\cdot\cdot\cdot}{}_{\mathrm{III}}} + \ldots nC_{M^{\cdot}{}_n} \ldots n \text{ charges}$$
$$= C_{A^{\prime}{}_{\mathrm{I}}} + 2C_{A^{\prime\prime}{}_{\mathrm{II}}} + 3C_{A^{\prime\prime\prime}{}_{\mathrm{III}}} + \ldots nC_{A^{\prime}{}_n} \ldots n \text{ charges}, \quad \ldots\ldots(152)$$

where $C_{M^{\cdot}{}_{\mathrm{I}}}$ stands for the concentration of monovalent cations and $C_{A^{\prime}{}_{\mathrm{I}}}$ for the concentration of monovalent anions and so on. As hydrogen and hydroxyl ions at reactions not far removed from the neutral point only occur in very small concentrations we can neglect them as terms in the above equation.

[1] D. D. van Slyke [1921, 1] has recently reviewed the question of the combination of CO_2 in blood from the theoretical as well as from the clinical standpoint. Those interested are referred to the original articles which only became known to me after the following had been written.

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$$= C_{A'}{}_{\text{I}} + 2C_{A''}{}_{\text{II}} + 3C_{A'''}{}_{\text{III}} + \ldots nC_{A'}{}_{n} \ldots n \text{ charges}, \quad \ldots\ldots(152)$$

where $C_{M^\cdot}{}_{\text{I}}$ stands for the concentration of monovalent cations and $C_{A'}{}_{\text{I}}$ for the concentration of monovalent anions and so on. As hydrogen and hydroxyl ions at reactions not far removed from the neutral point only occur in very small concentrations we can neglect them as terms in the above equation.

[1] D. D. van Slyke [1921, 1] has recently reviewed the question of the combination of CO_2 in blood from the theoretical as well as from the clinical standpoint. Those interested are referred to the original articles which only became known to me after the following had been written.

In a solution in which only strong acids and their salts are present combined CO_2 cannot exist as there are no cations to counterbalance the bicarbonate ions. But in a solution containing a free base the hydroxyl ions will react thus:

$$CO_2 + OH' = HCO'_3.$$

Under the given conditions this reaction practically proceeds quantitatively. In such a solution the combination of CO_2 will, as is well known, be independent of the pressure (if monocarbonate is not formed).

If a weak base or the salt of a weak acid is present in a solution, CO_2 will be combined in amounts varying with the CO_2 pressure if the reaction is suitable.

For a weak monovalent acid, as shown in chapter I, the following equation is valid:

$$\frac{a_{H'} \times a_{A'_I}}{a_{HA'_I}} = K_{HA_I}. \quad \text{.....(153)}$$

On saturation the following reaction

$$CO_2 + H_2O + A'_I \rightleftarrows HCO'_3 + HA_I \quad \text{.....(154)}$$

proceeds in such a way that a part of A'_I is substituted by HCO'_3 until the equilibrium

$$K_1 \frac{a_{A'_I}}{a_{HA_I}} = \frac{a_{HCO'_3}}{a_{H_2CO_3}} K_{HA_I} \quad \text{.....(155)}$$

is reached.

If a weak monovalent base is present in a solution the reaction

$$CO_2 + M_I\,OH \rightleftarrows M'_I + HCO'_3 \quad \text{.....(156)}$$

proceeds until the following equilibrium is reached:

$$\frac{a_{M'_I}}{a_{M_IOH}} \times \frac{K_W}{K_{M_IOH}} = K_1 \frac{a_{HCO'_3}}{a_{H_2CO_3}}. \quad \text{.....(157)}$$

The base is thus dissociated with the production of cations and bicarbonate ions. When equilibrium is attained an amount of bicarbonate ions will be present in the solution equivalent either to the cations the dissociation of which does not vary with the reaction (which before equilibrium were balanced by other anions the dissociation of which varies with the reaction) or to newly formed cations the dissociation of which varies with the reaction. The amount of combined CO_2 therefore becomes a measure of the *available cation concentration*. D. D. van Slyke and his collaborators have used the term "alkali reserve" for this amount (in serum) and E. Jarlöv has employed the almost analogous expression "available alkali." The reason I consider these expressions should be given up is because of the possibility of misunderstanding them. Thus Parsons, Davies, Haldane and Kennaway have erroneously assumed that in the "maximum" amount of CO_2 combined we have a measure of the difference between the cations and anions the dissociation of which does not vary with the reaction.

In order to illustrate the above I have performed experiments with sodium hydroxide solutions to which NaCl and phosphate solutions were added. The experiments will be found in Table LI and graphically in Fig. 15.

Table LI.

mm. Hg CO_2 1	Vols % CO_2 combined 2	p_H calc. 3	$a_{H^+} \times 10^{-7}$ calc. 4	With equation (171): Vols. % CO_2 combined 5	With equation (171): Diff. Vols. % 6 (2—5)	p_H calc. 7	With equation (172): Vols. % CO_2 combined 8	With equation (172): Diff. Vols. % 9 (2—8)	With equation (180): Vols. % CO_2 combined 10	With equation (180): Diff. Vols. % 11 (2—10)
I. 30 cc. *m*/15 KH_2PO_4 + 50 cc. 0·04941*n* NaOH + H_2O to 100 cc. + 0·42 g. NaCl. 20°.										
7·0	16·1	7·585	0·260	17·0	−0·9	7·608	17·6	−1·5	15·9	+0·2
12·7	19·4	7·407	0·385	19·9	−0·5	7·418	20·2	−0·8	19·7	−0·3
23·4	23·5	7·225	0·596	23·7	−0·2	7·229	23·8	−0·3	23·6	−0·1
46·4	28·0	7·004	0·990	28·7	−0·7	7·015	29·1	−0·9	28·3	−0·3
76·5	31·9	6·844	1·43	32·7	−0·8	6·854	33·2	−1·3	32·4	−0·5
172·3	38·4	6·571	2·68	39·3	−0·9	6·582	39·8	−1·4	37·6	+0·8
745·0	48·7	6·038	9·15	48·8	−0·1	6·039	49·2	−0·5	49·1	−0·4
II. 6 cc. *m*/15 Na_2HPO_4 + 24 cc. *m*/15 KH_2PO_4 + 50 cc. 0·04941*n* NaOH + H_2O to 100 cc. + 0·42 g. NaCl. 19°.										
8·6	49·2	7·954	0·111	49·4	−0·2	7·956	49·4	−0·2	43·1	+6·1
14·4	51·0	7·746	0·180	51·2	−0·2	7·747	51·3	−0·3	47·8	+3·2
20·2	52·1	7·608	0·247	52·9	−0·8	7·614	52·8	−0·7	50·7	+1·4
26·7	53·5	7·498	0·318	54·3	−0·8	7·504	54·6	−1·1	53·2	+0·3
47·3	57·7	7·283	0·521	58·4	−0·7	7·288	58·6	−0·9	57·9	−0·2
82·5	62·0	7·073	0·846	63·0	−1·0	7·080	63·0	−1·0	62·7	−0·7
436·4	77·5	6·446	3·58	78·8	−1·3	6·453	78·1	−0·6	76·4	+1·1
750·2	80·6	6·228	5·92	82·9	−2·3	6·240	82·1	−1·5	81·4	−0·8
III. 30 cc. *m*/15 Na_2HPO_4 + 50 cc. 0·04941*n* NaOH + H_2O to 100 cc. + 0·42 g. NaCl. 20°.										
5·2	56·3	8·240	0·0576	56·7	−0·4	8·242	56·8	−0·5	48·3	+8·0
12·5	58·8	7·878	0·132	58·8	0·0	7·878	58·8	0·0	54·1	+4·7
19·6	60·4	7·694	0·202	60·7	−0·3	7·696	60·8	−0·4	58·1	+2·3
25·2	62·4	7·599	0·252	61·8	+0·6	7·595	61·9	+0·5	60·2	+2·2
43·5	65·4	7·383	0·415	65·4	0·0	7·383	65·4	0·0	64·9	+0·5
81·0	70·1	7·142	0·721	70·3	−0·2	7·144	70·4	−0·3	70·1	0·0
186·2	77·4	6·824	1·50	78·1	−0·7	6·828	78·1	−0·7	77·0	+0·4
414·4	84·2	6·513	3·07	85·5	−1·3	6·520	85·7	−1·5	83·8	+0·4
731·5	87·5	6·283	5·21	90·1	−2·6	6·296	90·2	−2·7	88·8	−1·3
IV. 24 cc. *m*/15 Na_2HPO_4 + 6 cc. *m*/15 KH_2PO_4 + 0·0243*n* Na_2CO_3 to 100 cc. + 0·85 g. NaCl. The two first experiments at 18°, the others at 19°.										
6·9	32·8	7·835	0·146	33·3	−0·5	7·841	33·4	−0·6	27·6	+5·2
10·4	34·1	7·673	0·212	34·9	−0·8	7·683	35·0	−0·9	31·4	+2·7
21·1	37·6	7·416	0·384	38·6	−1·0	7·427	38·7	−1·1	37·3	+0·3
42·7	42·4	7·162	0·689	43·6	−1·2	7·174	43·9	−1·5	43·1	−0·7
85·3	48·6	6·921	1·20	49·3	−0·7	6·927	49·5	−0·9	48·7	−0·1
221·8	57·1	6·575	2·66	58·0	−0·9	6·582	58·3	−1·2	56·7	+0·4
414·0	63·0	6·347	4·50	63·0	0·0	6·347	63·0	0·0	62·0	+1·0
747·3	67·0	6·117	7·63	66·8	+0·2	6·117	66·6	+0·4	67·3	−0·3
747·3	66·6	6·115	7·68	66·8	−0·2	6·114	66·8	−0·2	67·3	−0·7
V. 24 cc. *m*/15 Na_2HPO_4 + 6 cc. *m*/15 KH_2PO_4 + 50 cc. 0·04941*n* NaOH + H_2O to 100 cc. + 0·85 g. NaCl. 19°.										
10·0	49·9	7·859	0·138	50·1	−0·2	7·861	50·1	−0·2	47·4	+2·5
22·7	53·0	7·529	0·296	53·7	−0·7	7·534	53·9	−0·9	53·8	−0·8
47·7	57·8	7·244	0·570	58·9	−1·1	7·252	59·1	−1·3	59·3	−1·5
85·2	62·3	7·023	0·945	63·9	−1·6	7·036	64·1	−1·8	63·6	−1·3
178·6	68·5	6·744	1·80	70·9	−2·4	6·759	71·2	−2·7	69·0	−0·5
375·2	76·9	6·473	3·37	77·6	−0·7	6·476	77·7	−0·8	74·2	+2·7
743·2	81·0	6·198	6·34	82·6	−1·6	6·207	82·6	−1·6	79·5	+1·5

In the first three solutions 0·42 % NaCl was present and 50 cc. 0·04941n NaOH in 100 cc. solution. In the last two solutions 0·85 % NaCl was present and in the first of this series 70 cc. 0·0243n Na_2CO_3 in 100 cc. In the last, NaOH was of the same concentration as in the first three. In all the solutions there was also 0·02m phosphate (30 cc. of S. P. L. Sörensen's phosphate mixtures to 100 cc. solution), the alkali content of the solutions being varied by adding different amounts of primary and secondary phosphate. The experiments were performed as usual (with the small saturator). In the first column of the table the CO_2 tension is given; in the second column the combined CO_2 in vols. %; in the third column $p_{H'}$ calculated in the usual way, the values for K'_1 given later being used; the fourth column contains $a_{H'}$.

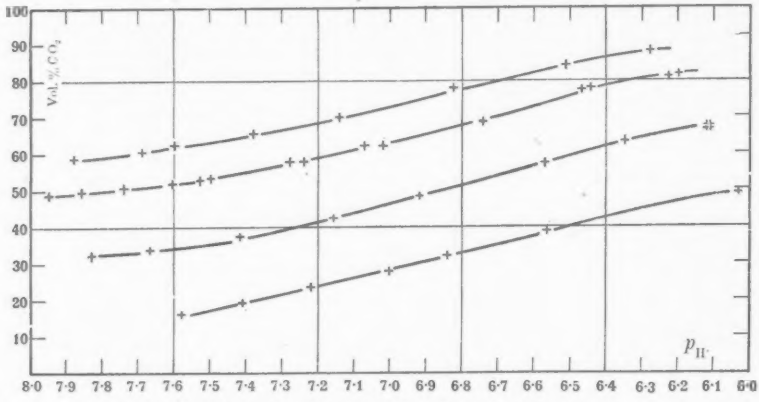

Fig. 15. The combination of CO_2 in 0·02m phosphate solution.

We will let C_I stand for the difference between the total cations and total anions the dissociation of which does not depend upon the reaction. The value for K'_1 employed is calculated by equations (139) and (143). The first dissociation of phosphoric acid

$$H_3PO_4 \rightleftarrows H^{\cdot} + H_2PO'_4 \quad \text{.....(158)}$$

progresses, under the conditions of the experiment, from left to right completely, the dissociation constant according to Abbott and Bray [1909] being $10^{-2\cdot0}$. The third dissociation of phosphoric acid under the same conditions does not take place at all, that is to say the reaction

$$HPO''_4 \rightleftarrows H^{\cdot} + PO'''_4 \quad \text{.....(159)}$$

proceeds completely from right to left, the dissociation constant according to Abbott and Bray being $10^{-12\cdot5}$. The only significant reaction therefore is

$$H_2PO'_4 \rightleftarrows H^{\cdot} + HPO''_4. \quad \text{.....(160)}$$

At a given CO_2 tension the following equilibrium holds for (160):

$$a_{H'} = K'_1 \frac{P_{CO_2}\alpha}{7{\cdot}60B} = k_2 \frac{C_{H_2PO'_4} F_a(H_2PO'_4)}{C_{HPO''_4} F_a(HPO''_4)}. \quad \text{.....(161)}$$

(An almost analogous equation was evolved by L. J. Henderson as early as 1908.)

If we now put

$$k_2 \frac{F_a(H_2PO'_4)}{F_a(HPO''_4)} = k'_2 \quad \text{.....(162)}$$

we get

$$a_{H'} = K'_1 \frac{P_{CO_2}\alpha}{7{\cdot}60B} = k'_2 \frac{C_{H_2PO'_4}}{C_{HPO''_4}}. \quad \text{.....(163)}$$

The total phosphate concentration is expressed by

$$C_{Ph} = C_{H_2PO'_4} + C_{HPO''_4}. \quad \text{.....(164)}$$

The charge expressed in normalities corresponding to the phosphates then becomes

$$uC_{Ph} = C_{H_2PO'_4} + 2C_{HPO''_4}. \quad \text{.....(165)}$$

From (163), (164) and (165) we get

$$u = \frac{15{\cdot}20Bk'_2 + K'_1 P_{CO_2}\alpha}{7{\cdot}60Bk_2 + K'_1 P_{CO_2}\alpha} \quad \text{.....(166)}$$

or

$$u = \frac{2k'_2 + a_{H'}}{k'_2 + a_{H'}}. \quad \text{.....(167)}$$

Further, C_I is defined by

$$C_I = C_{HCO'_3} + C_{H_2PO'_4} + 2C_{HPO''_4} \quad \text{.....(168)}$$

and therefore from (168) and (165)

$$B = (C_I - uC_{Ph})\,2226. \quad \text{.....(169)}$$

From (169) and (166) we obtain

$$B^2 + B\frac{K'_1 P_{CO_2}\alpha + 2226 \times 7{\cdot}60k'_2(2C_{Ph} - C_I)}{7{\cdot}60k_2} + \frac{K'_1\,2226\,P_{CO_2}\alpha\,(C_{Ph} - C_I)}{7{\cdot}60k'_2} = 0. \quad \text{...(170)}$$

Substituting in this the constants given in Table LI *a* we get, for the first series

$$B = 5{\cdot}2 - 0{\cdot}217\,P_{CO_2} + \sqrt{0{\cdot}0471\,P^2_{CO_2} + 21{\cdot}5\,P_{CO_2} + 27{\cdot}0};$$

for the second series

$$B = 23{\cdot}0 - 0{\cdot}230\,P_{CO_2} + \sqrt{0{\cdot}0529\,P^2_{CO_2} + 31{\cdot}6\,P_{CO_2} + 529};$$

for the third series

$$B = 27{\cdot}5 - 0{\cdot}226\,P_{CO_2} + \sqrt{0{\cdot}0511\,P^2_{CO_2} + 32{\cdot}6\,P_{CO_2} + 756};$$

for the fourth series at 18°

$$B = 14{\cdot}5 + 0{\cdot}252\,P_{CO_2} + \sqrt{0{\cdot}0635\,P^2_{CO_2} + 29{\cdot}8\,P_{CO_2} + 210};$$

at 19°

$$B = 14{\cdot}5 + 0{\cdot}248\,P_{CO_2} + \sqrt{0{\cdot}0615\,P^2_{CO_2} + 29{\cdot}3\,P_{CO_2} + 210};$$

for the fifth series

$$B = 23{\cdot}0 + 0{\cdot}250\,P_{CO_2} + \sqrt{0{\cdot}0626\,P_{CO_2} + 33{\cdot}9\,P_{CO_2} + 529}. \quad \text{.....(171)}$$

Table LI *a*. Constants used in the calculation of (171).

Series	Total cation concentration	C_I	K'_1	C_{Ph}	k'_2	Temp.
I	0·1165	0·0447	$10^{-6\cdot286} = 5{\cdot}18 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	19
II	0·1325	0·0607	$10^{-6\cdot278} = 5{\cdot}36 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	20
III	0·1365	0·0647	$10^{-6\cdot268} = 5{\cdot}40 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	19
IV (18°)	0·1940	0·0530	$10^{-6\cdot244} = 5{\cdot}70 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	18
IV (19°)	0·1940	0·0530	$10^{-6\cdot239} = 5{\cdot}77 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	19
V	0·2027	0·0607	$10^{-6\cdot235} = 5{\cdot}82 \times 10^{-7}$	0·02	$10^{-6\cdot86} = 1{\cdot}38 \times 10^{-7}$	19

k'_2 is taken as $10^{-6\cdot86}$, which has been very accurately determined by S. P. L. Sörensen [1909–1910] in a mixture of equal parts of $m/15$ KH_2PO_4 and $m/15$ Na_2HPO_4. It is probable that the constant in our case only differs slightly from this value. In solutions of 0·42 % NaCl, Y is taken as 6, in other solutions, as 8. The results of the calculations will be found in Table LI in the fifth column; in the sixth column the differences between the values found and calculated are given. If we put aside the experiments with the highest CO_2 tensions (which are subject to the largest experimental error), it will be seen the agreement is as good as could be expected on the whole, but it should be noted the calculated value is always a little greater than that found by experiment. The cause of this is not quite clear but it may be pointed out that the agreement would have been very nearly ideal if k'_2 was taken as $10^{-6\cdot80}$.

Now that we have effected these calculations let us turn again to the equations (171). It will immediately be obvious that it is difficult to form a clear conception of the course of the five carbonic acid combination curves if we only have these equations to go by, but it will be simple if from (167) and (169) we evolve the equation

$$B = \left(C_I - C_{Ph}\frac{2k'_2 + a_{H^\cdot}}{k_2 + a_{H^\cdot}}\right) 2226. \quad \text{.....(172)}$$

This equation indicates that all the CO_2 combination curves in solutions which contain the same phosphate concentration run parallel with one another if they are drawn in a right angled coordinate system where the apparent hydrogen ion activity, or a value which contains no other variable than this, is placed along one axis and the combined CO_2 along the other. If the combined CO_2 is expressed in vols. % the distance between the two curves projected on the vols. % axis will be the difference between the $C_I \times 2226$ (vols. %) of the two solutions.

The above reactions are of general interest in that, in any similar solution whatever we have

$$B = (C_I - C_V f(a_{H^\cdot}))\, 2226, \quad \text{.....(173)}$$

where C_V is the concentration of the electrolytes of which the dissociation varies with the reaction, and where C_I and $C_V f(a_{H^\cdot})$ can have positive or negative values. Even though we do not know C_I, C_V, or $f(a_{H^\cdot})$ we can, under the given assumptions, be certain of the shift in the CO_2 combination curve if C_I varies on the addition of alkali (or acid), the volume of the system being constant. If for instance C_I is increased to $C_I + b$ we get

$$B_I = (C_I + b - C_V f(a_{H^\cdot}))\, 2226. \quad \text{.....(174)}$$

Subtracting (173) from this we obtain

$$B_I - B = 2226b, \quad \text{.....(175)}$$

which is the algebraic expression of the fact that any CO_2 combination curve will be shifted parallel with itself in an $a_{H^\cdot}$ – vols. % CO_2 graph provided adsorption combinations of CO_2, or CO_2 compounds in the form of complex

bodies (*e.g.* carbamino-acids), are not formed. The distance between the curves is then $2226b$ vols. %.

If the CO_2 in a solution can be adsorbed or combined in the complex manner indicated above and if these combinations are reversible, the amount combined will be a function of the CO_2 pressure. The general expression for the combination will be

$$B = (C_I - C_V f(a_{H^\cdot}) - f_1(P_{CO_2}))\, 2226, \quad \text{.....(176)}$$

and the difference in CO_2 combined in a solution before and after the addition of alkali at the same reaction will therefore, from analogy with (175), be

$$B_I - B = 2226b - 2226\,(f_1(P_{CO_2}) - f_1(P_{CO_{2_I}})). \quad \text{.....(177)}$$

In this equation the amount

$$-\,2226\,(f_1(P_{CO_2}) - f_1(P_{CO_{2_I}}))$$

will always be positive because the combination of CO_2 must increase with the CO_2 tension in the given conditions and in order to produce the same reaction a higher CO_2 tension will be necessary after the addition of alkali than before (cf. equation (95)).

It is thus possible to state in general that a CO_2 combination curve, in which the combined CO_2 is set off along the one axis and an expression for the active reaction along the other axis, will be shifted on the addition of alkali in such a way:

(1) that the distance between the original curve and the new curve is always equal to the combination of CO_2 corresponding to the added amount of alkali provided that the variable combination in each curve is only a function of the reaction;

(2) that the distance between the original and new curves will increase with the apparent hydrogen ion activity provided that the variable combination is also a function of the CO_2 tension;

(3) that the distance between the two curves can never be less than the combined CO_2 corresponding to the added alkali.

We will now revert to the phosphate solutions again. In the eighth column of the table the combined CO_2 expressed in vols. % will be found, calculated by (172) with the help of the apparent hydrogen ion activity in column 4 reckoned directly from the experiments. The agreement is, as expected, good. In Fig. 15 the combined CO_2 is plotted as ordinates and

$$-\log a_{H^\cdot} = p_{H^\cdot}$$

as abscissae, however on a hundred times greater scale. In such a coordinate system, as explained, the course of the curves will be parallel. Now it appears from the figure that the CO_2 combination curves are straight lines for a long portion of their courses, and we will therefore, as an example of what is to follow, calculate these sections of the curves by the method of least squares, using the figures in columns 2 and 3, and include all experiments in which

p_H is less than 7·59. Equations (141) and (142) are employed in the calculation and the mean error of the constants is estimated by the equation

$$M_x = M_y = \frac{M}{\sqrt{n(\Sigma a^2 - (\Sigma a)^2)}}, \quad \text{.....(178)}$$

where

$$M = \sqrt{\frac{\Sigma a^2}{n-2}}, \quad \text{.....(179)}$$

Σa being the sum of the squares of the deviations of the found and calculated values and n the number of experiments. The equation of the straight line referred to above is

$$x \log a_H + y = B, \quad \text{.....(180)}$$

where x is the tangent to the angle made by the line with the axis of the abscissa, measured in the first or third quadrant, and y is the part of the ordinate axis cut off by the line. The calculation gives

Series	x	y	M	No. of experiments
I	21·460 ± 0·057	178·65 ± 0·057	0·51	7
II	22·139 ± 0·17	219·15 ± 0·17	0·91	5
III	21·720 ± 0·16	225·24 ± 0·16	0·87	5
IV	23·076 ± 0·077	208·41 ± 0·077	0·68	7
V	19·301 ± 0·27	199·13 ± 0·27	1·9	6

It will be seen that a determination of the error indicates that x is different n the various series, but as it has been proved already by calculations that the different curves are really parallel, allowing for experimental error, we cannot attribute any real significance to the mean error calculated by this method in the present and similar cases. There are several reasons for this rather unsatisfactory result. In the first place the number of experiments in each series, within the range of reaction employed, is far too few to permit of an accurate determination of the error; in the second place the points determined are not regularly distributed over the various sections of the curve, in fact they do not all cover exactly the same range of reaction, which is of importance as it will be shown the portion of the curve in question is not in reality a straight line. For the appreciation of what is to follow it will be necessary to examine this a little more closely.

By equation (172) it is easy to estimate the true slope of the curve. In the second column of the following table the combinations of CO_2 in a 0·02n phosphate solution of p_H varying from 7·50 to 6·20 are given, 2226 C_I = 52·3 vols. %, the combined CO_2 at p_H 7·50 being here put equal to 0. In the third column will be found the tangent to the straight lines (angle of inclination which is x) which join the nearest points together two by two. If the point referring to p_H 7·50 is joined with that referring to p_H 6·20 the tangent to the angle of inclination of the line is 21·6. In the fourth column will be found the amount of combined CO_2 expressed in vols. %, which, according to a calculation based on the straight line smoothing formulae (141) and (142) from the figures in columns 1 and 3, should be found combined at the given p_H when the best constants are used. These constants are

$$x = 22{\cdot}719 \pm 0{\cdot}021, \quad y = 169{\cdot}77 \pm 0{\cdot}021.$$

In the fifth column the differences between the CO_2 combinations calculated by (172) and by the smoothing are given. The standard deviation is calculated to 0·45 vols. %.

Table LI *b*.

p_H	Vols. % CO_2 combined. Calculated by (172)	x	Vols. % CO_2 combined. Algebraic smoothing	Difference vols. %
1	2	3	4	5 (2–4)
7·50	0·0		−0·6	+0·6
		17		
7·40	1·7		1·7	0·0
		19		
7·30	3·6		3·9	−0·3
		21		
7·20	5·7		6·2	−0·5
		23		
7·10	8·0		8·5	−0·5
		24		
7·00	10·4		10·7	−0·3
		25		
6·90	12·9		13·0	−0·1
		26		
6·80	15·5		15·3	+0·2
		25		
6·70	18·0		17·6	+0·4
		24		
6·60	20·4		19·8	+0·6
		22		
6·50	22·6		22·1	+0·5
		21		
6·40	24·7		24·4	+0·3
		18		
6·30	26·5		26·6	−0·1
		16		
6·20	28·1		28·9	−0·8

If column 5 is examined it will be observed that the differences change their sign three times, that is to say the straight line cuts the true curve (calculated by (172)) three times. If it should happen, in the determination of a similar CO_2 combination curve, that points were obtained at these places of intersection, it would naturally follow that even an ideal method of analysis would be unable to show that the curve departed from a straight line. It will be realised that a very considerable number of experimental points are required to determine the true course of the curve and that in using the smoothing method of calculation one must be exceedingly cautious about one's conclusions.

As mentioned the constant x was determined by the smoothing method to be $22{\cdot}719 \pm 0{\cdot}021$. If this is compared with the constants found by calculation from the experiments it will be seen that only that from series II appears to be of the same magnitude, which is another plea for the contention that the mean error of the constants is really too sharply defined.

There is still another point in relation to the smoothing method to be touched upon. It is not possible by this method in the form which is used here with the aid of the mean error of the constants to determine the mean error from a single point on the curve. This is due to the choice of the zero point of the coordinate system, because this lies, as will be seen, far outside the experimentally determined points. The reason I have given the numbers for the constants correct to the first decimal place in spite of the objections

put forward relating to the mean error, is in order to show them with all the figures used in the calculations and the mean error is therefore only employed roughly to demonstrate the uncertainty of the constants.

It is accepted that a large part of the reversibly combined CO_2 of the blood is present as the bicarbonate ion, the haemoglobin and other protein substances acting as ampholytes. The objections against this view which have been put forward in the last few years have been to some extent negatived in previous chapters and will also be questioned at the end of the present one. It can therefore be concluded that no general condition for the combination of CO_2 in the blood can be formulated which cannot be traced back to an equation of the form of (173) or (176). Provided no CO_2 is adsorbed or combined in a complex manner (173) will be the correct equation.

In 1920 T. R. Parsons put forward a mathematical treatment of the CO_2 combination in the blood but it seems to me he has made a mistake on this point. Firstly Parsons assumed that haemoglobin is a monobasic acid, each haemoglobin molecule however encompassing several such groups. Secondly he assumed that in blood especially there were so many more cations than anions the dissociation of which did not vary with the reaction, that they were able just to neutralise the haemoglobin acid. Thirdly he believed that in the "maximum" of combined CO_2 we have a measure of the concentration of the haemoglobin acid.

The first of these assumptions is quite uncertain; the second is undoubtedly erroneous, because the blood can expel CO_2 from alkaline carbonate as Pflüger [1864] was the first to show. The third assumption is incorrect as there is no maximal CO_2 combination in blood at atmospheric CO_2 tensions. Parsons' final relation evolved as it is from faulty assumptions has no real significance and it is only one of many instances that nearly every equation with a sufficient number of constants can be brought into agreement with an observed fact when the constants chosen are the best possible.

At present there seems to be no possibility—contrary to what was the case with the phosphate experiments—of establishing an *a priori* relation between $a_{H^\cdot}$ and the combination of CO_2 with the blood proteins. There is no other course open therefore than to find the simplest empirical relation between these values.

In the previously mentioned paper of Hasselbalch and Warburg some CO_2 combination curves for blood and serum are given in a $p_{H^\cdot}$—vols. % curve. They are straight lines. This fact appears to have been quite overlooked, but I believe the appreciation of this phenomenon will be of far-reaching importance in the physiology of respiration. In this work I shall content myself with more thoroughly demonstrating the relation and employ it for elucidating the question of the existence of complex CO_2 combinations as well as some other problems.

In tables LII—LVII (pp. 287–290) the combination of CO_2 at various CO_2 tensions in a number of blood samples from different people and animals is given. In the last column but one the results of the algebraic smoothing are recorded, the CO_2 combination curve in the $p_{H^\cdot}$—vols. % combined CO_2 chart being taken as a straight line.

It is therefore assumed that

$$x \log a_{H^+} + y = B \quad \text{.....(181)}$$

or

$$-xp_{H^+} + y = B. \quad \text{.....(182)}$$

That these relations, within the experimental error, are really true seems to follow from the curves and tables, but I have further investigated some rather extensive unpublished material dealing with the CO_2 combination curves in ox blood which has been elaborated of late years for another purpose in the laboratory of the Finsen Institute, without being able to find any sign of a departure from the relations. After what was said about the phosphate curves it will readily be understood I do not wish to urge that the sections of the curves being dealt with are actually straight lines, but it is asserted the deviations from a straight line are small compared with the variations in the combination of CO_2 at different apparent hydrogen ion activities.

In the calculation of $p_{H^+(s)}$ (130) was used. In the case of oxygenated human blood at 38° and oxygenated horse blood at room temperature the corrections given in Fig. 10 were added to the values found. In the case of reduced human blood at 38° and reduced horse blood at room temperature the correction corresponding to p_{H^+} (uncorrected $-$ 0·40) was added. In the case of Hasselbalch's [1916] "half reduced" blood 0·20 was subtracted from p_{H^+} (uncorrected), while 0·40 was subtracted from oxygenated ox blood at room temperature. Equation (130) was used without correction in the calculation of the other blood samples.

The correction curve corresponding to 18·5 vols. % combined CO_2 was used in the calculations relating to Haldane's blood, from Christiansen, Douglas and Haldane [1914], the 15 vols. % curve for Parsons' [1917] blood, the 20 vols. % curve for Davies' [1920] and Hasselbalch's [1916, 2] blood and the 25 vols. % curve for Joffe's [1920] and Warburg's blood[1]. In the calculations

[1] I have not attempted to discover how much combined O_2 was actually present in Davies', Haldane's, Joffe's and Parsons' blood but I have contented myself with estimating their p_{H^+}–CO_2 combination curves by means of the corrections mentioned because an error of 5 vols. % combined O_2 only causes a difference in the constant x of about 1. The reason of this is that I have not been able to obtain agreement between the O_2 determinations got by pumping out and the values obtained by Haldane's ferricyanide method. I originally became aware of this by comparing the relation between the O_2 combined by the blood and the volume of the blood corpuscles of the same blood. A. Norgaard and H. C. Gram and later H. C. Gram [1921] found about 38·5 vols. % combined O_2 in 100 cc. human blood corpuscles but V. Bie and P. Möller [1913] found about 45 vols. %, a similar quantity to that I have myself found. Later on, it appears, W. B. Cannon, J. Fraser and A. N. Hooper [1919], in oxygenated blood from patients suffering from shock, found about 43 vols. %. While Norgaard and Gram's haemoglobinometer was corrected by the ferricyanide method, Bie and Möller's apparatus was corrected by Fridericia by pumping out, just as my own results were obtained. Dr Marie Krogh was kind enough to determine the O_2 in some blood samples from man and horse with a Haldane haemoglobinometer which was very accurately calibrated by the ferricyanide method. She constantly found lower O_2 values than I did with the exhaust pump. I have no doubt whatever that too low readings are obtained under special circumstances with Haldane's ferricyanide method as shown lately by van Slyke and Stadie [1921] and I hope soon to take up this question again. F. Müller [1904] previously found the same for dog blood but considered it only applied to quite fresh blood and under unfavourable circumstances.

relating to ox and horse blood the curve which most nearly corresponded to the O_2 determinations was employed.

The value used in the calculations for $p\lambda_{(s)} + \log \Phi_s (CO_2)$ with human, ox, and horse blood at 38° was 6·190; at 37°, 6·185; at 17°, 6·334; at 18°, 6·327; at 19°, 6·320. In the case of pig and pigeon blood at 38°, 6·20 was used; with dog and rabbit blood at 19° and 18·5°, 6·35. With haemolysed blood equation (134) was employed with the following constants: at 17°, 6·325 was employed; at 18°, 6·320; at 19°, 6·315. With serum equation (121) was used and as constants at 38°, 6·165 was used; at 19°, 6·286; at 20°, 6·281; at 21°, 6·274. The results of these calculations and of the smoothing on the curves for some of the experiments carried out in the laboratory of the Finsen Institute will be found in Tables LII, LIII and LVIII and in Figs. 16, 17, 19, 20 and 21. The combination of CO_2 in *human, pigeon, horse, dog, rabbit, pig and ox blood* and in some sera can therefore be expressed by an equation of the form of (181) or (182).

It will be observed, as already mentioned, that all the curves determined in this laboratory are straight lines, allowing for experimental error. In the case of a number of the serum curves however the point corresponding to the highest CO_2 tension is too low. This seems to me only to be an indication that equilibrium had not been quite reached in these experiments as at the time I undertook them I was not aware of the deficiency of the small saturator. I have omitted these experiments in the calculus of smoothing, and in the tables this is denoted by a line drawn through them. It will be easily seen without a special note which experiments are included and which rejected. Those portions of the curves which correspond to the rejected experiments are drawn with a dotted line. In one of the tables an experiment has been excluded because it seems to be subject to an unusually large error. This experiment is put in parentheses.

The experiments with very low tensions were carried out with the large saturator electrodes using the technique described in chapter IV.

In Table LIV I have performed similar calculations for Haldane's blood (taken from Christiansen, Douglas and Haldane's [1914] paper) and in Table LVI for Davies' blood (taken from Davies, Haldane and Kennaway's [1920] paper). The curves are given in Fig. 18.

It will be noticed the CO_2 combinations corresponding to the most alkaline values are too high both in oxygenated and reduced blood—in Davies' blood the most alkaline values are too high and the most acid too low. I believe I am right in saying these deviations from a straight line are due to the fact that the technique of saturation employed is not applicable at high and low CO_2 tensions. The experiments just recorded show this and I myself had a similar failure before I became aware of the relatively great difficulties of saturation. Davies, Haldane and Kennaway's mistake in thinking the combination of CO_2 in the blood is maximal at the highest tensions is only due to equilibrium not being established. Calculations relating to J. Joffe's blood, Table LVII and Fig. 19 (from Joffe and Poulton's [1920] paper) also demonstrate

Table LII.

Pig blood (O_2-combination 21·1 vols. %). Half saturated with O_2, 38°. Calculated after K. A. Hasselbalch. $p\lambda_{(a)} + \log \Phi_a = 6{\cdot}20$ in every case.

mm. Hg CO_2	Vols. % CO_2 combined	$pH'_{(a)}$ calculated	Vols. % CO_2 combined calculated	Difference
8·4	21·9	7·75	20·8	+1·1
(11·4	22·7	7·64	26·5	−3·8)
15·7	28·6	7·60	28·6	0·0
30·4	36·8	7·42	37·9	−1·1
30·8	36·4	7·41	38·4	−2·0
53·1	46·3	7·28	45·2	+1·1
76·1	51·8	7·17	51·0	+0·8

$x = 51{\cdot}865 \pm 0{\cdot}5$. $y = 422{\cdot}76 \pm 0{\cdot}5$. $M = 1{\cdot}4$.

Pigeon blood (O_2-combination 23·7 vols. %). Half saturated with O_2, 38°. Calculated after K. A. Hasselbalch. $p\lambda_{(a)} + \log \Phi_a = 6{\cdot}20$ in every case.

5·8	21·0	7·90	20·9	+0·1
18·3	35·0	7·62	35·1	−0·1
38·5	45·3	7·41	45·7	−0·4
75·0	56·2	7·21	55·9	+0·3

$x = 50{\cdot}774 \pm 0{\cdot}08$. $y = 421{\cdot}96 \pm 0{\cdot}08$. $M = 0{\cdot}4$.

Dog blood (O_2-combination 19·2 vols. %) totally reduced, 19°. $p\lambda_{(a)} + \log \Phi_a = 6{\cdot}35$ in every case.

2·2	21·8	8·27	22·1	−0·3
29·3	53·5	7·54	53·6	−0·1
56·4	62·4	7·32	63·1	−0·7
77·1	68·5	7·22	67·5	+1·0
127·5	76·0	7·05	74·8	+1·2
337·6	88·8	6·70	89·9	−1·1

$x = 43{\cdot}230 \pm 0{\cdot}1$. $y = 379{\cdot}58 \pm 0{\cdot}1$. $M = 1{\cdot}0$.

Rabbit blood totally reduced, 18·5°. $p\lambda_{(a)} + \log \Phi_a = 6{\cdot}35$ in every case.

16·0	48·6	7·76	44·4	+4·2
24·2	53·1	7·62	52·9	+0·2
41·5	63·0	7·46	62·6	+0·4
44·2	64·1	7·44	63·9	+0·2
81·7	73·8	7·23	76·6	−2·8
83·1	76·5	7·24	76·0	+0·5
368·0	107·0	6·74	106·4	+0·6

$x = 60{\cdot}838 \pm 0{\cdot}4$. $y = 516{\cdot}49 \pm 0{\cdot}4$. $M = 1{\cdot}5$.

Reduced horse blood (vols. % combined O_2, 22·6), 19°.

3·0	25·2	8·24	26·1	−0·9
22·0	56·2	7·68	55·7	+0·5
39·6	66·3	7·48	66·3	0·0
48·7	69·9	7·41	70·0	−0·1
80·2	78·2	7·24	79·0	−0·8
91·5	83·5	7·20	81·1	+2·4
484·7	112·2	6·59	113·4	−1·2

$x = 52{\cdot}908 \pm 0{\cdot}2$. $y = 462{\cdot}04 \pm 0{\cdot}2$. $M = 1{\cdot}3$.

Oxygenated horse blood (vols. % O_2, 25·4; 25, 1; 25, 2), 20°.

32·7	51·5	7·50	51·4	+0·1
75·2	69·4	7·24	69·4	0·0
194·5	91·1	6·92	91·4	−0·3
364·0	106·1	6·71	105·9	+0·2
558·4	116·2	6·56	116·2	0·0

$x = 68{\cdot}897 \pm 0{\cdot}06$. $y = 568{\cdot}17 \pm 0{\cdot}06$. $M = 0{\cdot}2$.

Oxygenated ox blood, 38°. Hasselbalch and Warburg. $p\lambda_{(a)} + \log \Phi_a = 6{\cdot}20$ in every case.

43·5	41·2	7·31	40·7	+0·5
93·6	54·3	7·10	54·9	−0·6
150·9	63·9	6·96	64·5	−0·6
203·9	70·1	6·87	70·4	−0·3
269·4	76·5	6·79	75·8	+0·7
361·8	83·4[1]	6·70	81·9	+2·5
692·9	101·9[1]	6·50	95·3	+6·6

[1] O_2 deficit.

$x = 67{\cdot}458 \pm 0{\cdot}03$. $y = 533{\cdot}81 \pm 0{\cdot}03$. $M = 0{\cdot}7$.

Table LIII.

K. A. Hasselbalch's half reduced blood, 38°. K. A. Hasselbalch [1916, 2][1].

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H^{\cdot}(s)}$ calculated	Vols. % CO_2 combined calculated	Difference
18·5	35·1	7·63	36·6	−1·5
22·4	36·7	7·56	40·6	−3·9
32·7	45·1	7·48	45·2	−0·1
40·9	48·2	7·41	49·3	−1·1
45·7	52·9	7·40	49·9	+3·0
50·8	57·1	7·39	50·4	+6·7
67·0	57·9	7·27	57·4	+0·5
80·7	61·3	7·21	60·9	+0·4
102·2	62·5	7·11	66·6	−4·1

$x = 57{\cdot}858 \pm 0{\cdot}9$. $y = 477{\cdot}98 \pm 0{\cdot}9$. M = 3·6.

E. J. Warburg's reduced blood, 38°.

13·6	27·7	7·66	25·9	+1·8
39·5	42·8	7·36	45·9	−3·1
74·6	56·5	7·20	56·5	0·0
109·2	64·7	7·09	63·8	+0·9
544·5	99·5	6·56	99·1	+0·4

$x = 66{\cdot}567 \pm 0{\cdot}5$. $y = 535{\cdot}79 \pm 0{\cdot}5$. M = 2·1.

[1] The experiments were performed with blood taken on three different days and were not specially designed for the determination of the CO_2 combination curve.

Table LIV.

J. S. Haldane's oxygenated blood, 37°.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H^{\cdot}(s)}$ calculated	Vols. % CO_2 combined calculated	Difference
2·6	14·9	8·15	3·8	+11·1
8·7	25·7	7·84	23·0	+ 2·7
18·9	35·8	7·64	35·5	− 0·3
37·7	48·6	7·46	46·6	+ 2·0
44·1	49·4	7·40	50·4	− 1·0
41·7	48·2	7·42	49·1	− 0·9
56·1	54·5	7·33	54·7	− 0·2
73·3	58·1	7·25	59·7	− 1·6
78·5	60·3	7·23	60·9	− 0·6
105·2	68·1	7·15	65·8	+ 2·3

$x = 62{\cdot}016 \pm 0{\cdot}5$. $y = 509{\cdot}32 \pm 0{\cdot}5$. M = 1·6.

J. S. Haldane's reduced blood, 37°.

2·15	15·3	8·24?	4·2	+11·1
7·75	30·3	7·94	23·6	+ 6·7
8·1	30·0	7·92	24·9	+ 5·1
17·9	40·4	7·69	39·8	+ 0·6
37·9	53·5	7·48	53·4	+ 0·1
58·2	60·5	7·34	62·5	− 2·0
74·2	65·3	7·27	67·0	− 1·7
78·7	69·9	7·27	67·0	+ 2·9
111·1	75·4	7·15	74·8	+ 0·6

$x = 64{\cdot}731 \pm 0{\cdot}7$. $y = 537{\cdot}60 \pm 0{\cdot}7$. M = 1·8.

Table LV.

T. R. Parsons' oxygenated blood, 37°.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(\omega)}$ calculated	Vols. % CO_2 combined calculated	Difference
5·7	21·3	7·93	21·4	−0·1
10·1	30·0	7·83	26·2	+3·8
19·6	34·4	7·59	37·6	−3·2
33·4	41·5	7·43	45·2	−3·7
37·4	43·4	7·40	46·6	−3·2
72·1	59·9	7·25	53·7	+6·2

$x = 47{\cdot}449 \pm 2{\cdot}4$. $y = 397{\cdot}70 \pm 2{\cdot}4$. $M = 5{\cdot}4$.

T. R. Parsons' reduced blood, 37°.

5·7	23·8	7·96	23·0	+0·8
8·1	27·4	7·87	27·7	−0·3
10·1	31·5	7·83	29·6	+1·9
19·6	39·9	7·64	39·8	+0·1
33·4	47·8	7·48	48·1	−0·3
37·3	47·3	7·42	51·3	−4·0
55·3	55·1	7·31	57·0	−1·9
72·1	64·4	7·26	59·6	+4·8

$x = 52{\cdot}319 \pm 0{\cdot}7$. $y = 439{\cdot}48 \pm 0{\cdot}7$. $M = 3{\cdot}0$.

Table LVI.

H. W. Davies' blood, 38°.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(\omega)}$ calculated	Vols. % CO_2 combined calculated	Difference
8·3	26·3	7·88	6·0	+20·3
9·02	29·6	7·90	4·3	+25·3
11·2	28·7	7·78	14·5	+14·2
19·3	35·5	7·63	27·2	+ 8·3
29·8	41·4	7·50	38·2	+ 3·2
38·9	46·4	7·44	43·3	+ 3·1
39·0	45·5	7·42	44·9	+ 0·6
39·1	43·1	7·39	47·5	− 4·4
46·6	47·3	7·35	50·9	− 3·6
51·3	53·2	7·36	50·0	+ 3·2
64·9	57·4	7·29	55·9	+ 1·5
68·5	60·0	7·28	56·8	+ 3·2
76·7	62·0	7·25	59·3	+ 2·7
89·9	65·7	7·20	63·5	+ 2·2
102·9	66·5	7·15	67·8	− 1·3
202·0	81·8	6·93	86·3	− 4·5
215·0	82·6	6·91	88·1	− 5·5
411·0	97·3	6·69	106·7	− 9·4
455·0	97·6	6·65	110·1	−12·5
497·0	95·7	6·60	114·4	−18·7

$x = 84{\cdot}657 \pm 0{\cdot}4$. $y = 673{\cdot}09 \pm 0{\cdot}4$. $M = 4{\cdot}8$.

Table LVII.

J. Joffe's defibrinated oxygenated blood, 4. viii.–28. xi. 1919.
Joffe and Poulton, Tables V and VII.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(\omega)}$ calculated	Vols. % CO_2 combined calculated	Difference
4·0	13·7	7·93	5·4	+ 8·3
5·0	16·8	7·92	6·1	+10·7
12·9	23·8	7·65	24·0	− 0·2
19·7	29·4	7·54	31·3	− 1·9
24·9	36·0	7·53	31·9	+ 4·1
34·6	40·0	7·42	39·3	+ 0·7
44·4	44·8	7·36	43·2	+ 1·6
50·2	44·1	7·30	47·2	− 3·1
58·0	47·6	7·26	49·9	− 2·3
65·3	52·6	7·25	50·5	+ 2·1
67·8	53·9	7·25	50·5	+ 3·4
82·2	56·0	7·17	55·8	+ 0·2
110·3	63·3	7·10	60·5	+ 2·8
149·0	62·6	6·96	69·8	− 7·2
156·0	67·1	6·97	69·1	− 2·0
157·0	70·3	6·98	68·4	+ 1·9
180·0	68·5	6·91	73·1	− 4·6
376·3	90·0	6·69	87·7	+ 2·3
610·0	100·3	6·53	98·3	+ 2·0

$x = 66{\cdot}345 \pm 0{\cdot}16$. $y = 531{\cdot}52 \pm 0{\cdot}16$. M = 3·2.

J. Joffe's defibrinated reduced blood, 4. viii.–9. x. 1920.
Joffe and Poulton, Table VII.

9·9	27·9	7·81	20·1	+7·8
15·0	34·4	7·72	26·3	+8·1
19·4	35·3	7·61	34·0	+1·3
27·6	40·1	7·50	41·6	−1·5
30·9	44·1	7·50	41·6	+2·5
36·8	47·9	7·45	45·1	+2·8
41·7	46·0	7·37	50·6	−4·6
47·0	50·4	7·36	51·3	−0·9
50·5	49·8	7·33	53·4	−3·6
55·7	52·9	7·31	54·8	−1·9
70·1	55·6	7·23	60·4	−4·8
76·8	62·1	7·23	60·4	+1·7
77·7	64·0	7·24	59·7	+4·3
92·7	67·6	7·19	63·1	+4·5

$x = 69{\cdot}475 \pm 0{\cdot}7$. $y = 562{\cdot}65 \pm 0{\cdot}7$. M = 3·6.

that the most alkaline values are too high but the acid values are correct. Parsons' [1917] blood gives values scattered irregularly about a straight line (Table LV and Fig. 19).

The constant x, which is the tangent to the angle the straight line makes with the axis of the abscissa measured in the first or third quadrant, is for

Davies'	oxygenated blood	...	84·7
Haldane's	,, ,, ...	...	62·0
,,	reduced ,, ...	...	64·7
Hasselbalch's	half oxygenated blood		57·9
Joffe's	oxygenated blood	...	66·3
,,	reduced ,,	...	69·5
Parsons'	oxygenated ,,	...	47·4
,,	reduced ,,	...	52·3
Warburg's	,, ,,	...	66·6

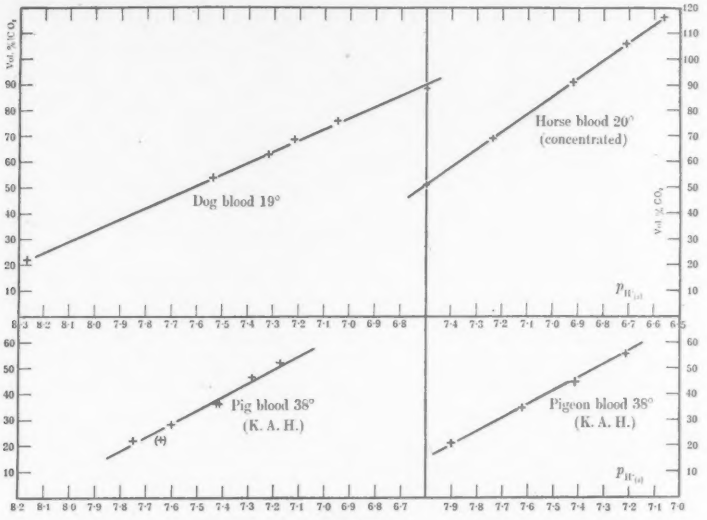
Dog blood 19°
Horse blood 20°
(concentrated)
Pig blood 38°
(K. A. H.)
Pigeon blood 38°
(K. A. H.)

Fig. 16.

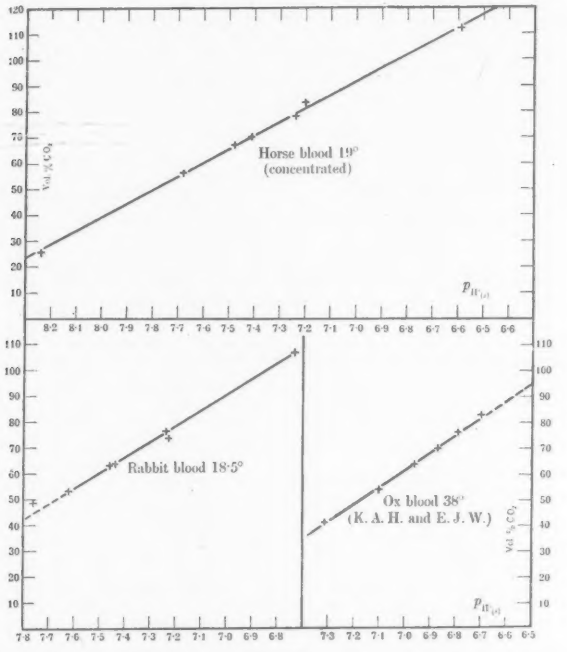
Horse blood 19°
(concentrated)
Rabbit blood 18·5°
Ox blood 38°
(K. A. H. and E. J. W.)

Fig. 17.

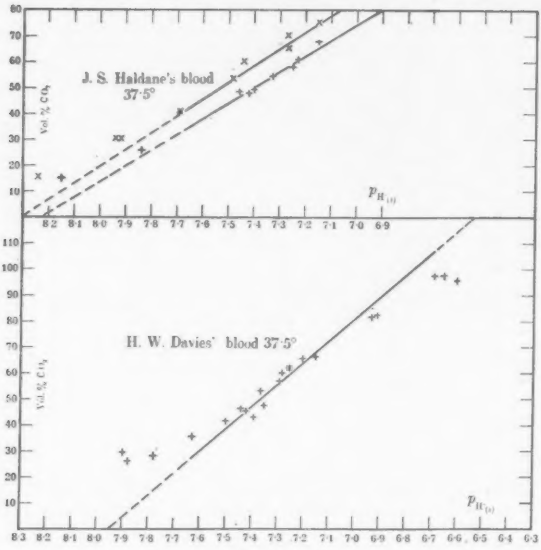
J. S. Haldane's blood
37·5°
Vol % CO_2
$p_{H_{(t)}}$
H. W. Davies' blood 37·5°
Vol % CO_2
$p_{H_{(t)}}$

Fig. 18.

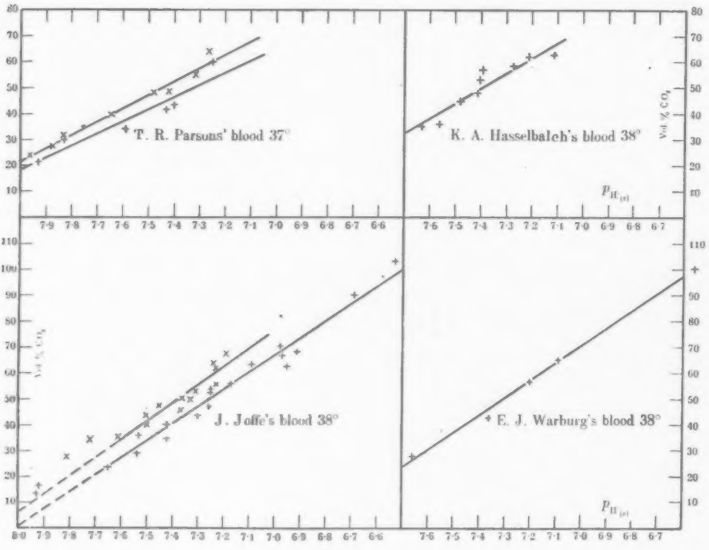
T. R. Parsons' blood 37°
K. A. Hasselbalch's blood 38°
Vol % CO_2
$p_{H_{(t)}}$
J. Joffe's blood 38°
E. J. Warburg's blood 38°
$p_{H_{(t)}}$

Fig. 19.

It will be observed in the first place that the constant varies from blood to blood. The greater part of the difference is undoubtedly due to variations in the amount of haemoglobin as Hasselbalch [1916, 2] has pointed out and as is evident from the experiments of Schmidt [1867], Zuntz [1867, 1868], Setschenow [1879], Jaquet [1892], Bohr [1905] and many others, but the examination of numbers of curves has shown that this cannot be the sole determining factor, as Peters, Jr. and Barr [1921] have also indicated from a rather different point of view (Peters and Barr's curves are constructed as P_{CO_2}—vols. % total CO_2 curves). It will also be seen that x is a little greater in reduced blood than in the corresponding oxygenated blood. The difference is certainly a real one and is of almost the same magnitude in Haldane's, Joffe's and Parsons' blood. L. J. Henderson [1920, 1] has recently demonstrated the same thing graphically and he has discussed the cause in a paper dealing with the influence of oxygen on the combined CO_2 of the blood. This paper seems to be of very considerable interest although I do not agree with the author in all his views but as I have not yet succeeded in quite clearing up several of the problems I shall only refer those interested to the original article.

By plotting a p_H·—vols. % combined CO_2 curve it is possible to form a good idea of the reliability of the curve. I have done this for a number of Haggard and Henderson's experiments and for some of Peters, Barr and Rule's also. It appears especially in the case of Haggard and Henderson's, that the technique is not so good as the authors believe and it seems to me problematical whether the accuracy is sufficient to draw any conclusion about the tension with which the blood is in equilibrium from the total amount of CO_2 in the blood, as these authors do. Haggard and Henderson's [1920, 1] discovery that there is no difference between the combined CO_2 in oxygenated and reduced oxalate blood, and that the combined CO_2 of the blood decreases irreversibly by blowing air through it vigorously, is presumably due to experimental error.

I must now discuss some experiments published in the last few years by H. Straub and Klothilde Meier [1918–1920]. They have unfortunately gained some recognition, *e.g.* by L. Michaelis[1] and by Parsons. But their results are so surprising, that Joffe and Poulton [1920], Peters, Barr and Rule [1921] for example have announced that they are sceptical. Straub and Meier's chief claim is that they have shown the combined CO_2 in the blood does not increase before a reaction of about p_H· 6·70 is reached when suddenly (in an interval of p_H· 0·01) an amount of CO_2 as great as the oxygen capacity is combined by the blood and then all further combination ceases. In haemo-

[1] Michaelis and Airila have recently [1921] shown by cataphoresis experiments that the change of haemoglobin varies uniformly with the reaction over a very large range of reaction and is thus greatly at variance with Straub and Meier's assertions about the ionisation of haemoglobin. The conclusion may be drawn from these interesting experiments that the combination of CO_2 in haemoglobin solutions can be represented by a curve which, if not a straight line, is a very close approximation thereto, in a p_H·–vols. % diagram over a very considerable range of reaction.

lysed blood the sudden change is at p_H 7·00 and is of the same extent. They believe serum is quite unable to combine with CO_2 in quantities varying with the pressure. Straub and Meier claim to have shown that various substances shift this sudden change backwards and forwards, and they have formed a theory which for extravagance is only surpassed by the obvious imperfection of their technique. With regard to these authors I cannot suppress my surprise that they really seem to be quite ignorant of the older, and as far as the present question is concerned, of the more recent literature on the combined CO_2 of the blood and it is to me completely incomprehensible that they have not paid more attention to their technique.

As an illustration of Straub and Meier's technique I will give one of their experiments [1920, 2, p. 250].

Experiment 9. 38°.

mm. Hg CO_2	Vols. % CO_2 total
16·8	15·1
50·2	19·6
72·6	30·6
153·3	38·7
41·4	35·9
64·0	34·9

The only possible explanation is, as the authors themselves have realised, that equilibrium has not been reached again at the low CO_2 tension. Instead of examining their technique they employ the experiment in propounding one of their extraordinary theories, and they fail to see that p_H cannot be calculated by Henderson and Hasselbalch's equation if equilibrium in the system has not been attained. The following similar experiment shows that equilibrium can easily be attained.

Ox blood slightly concentrated by centrifuging. Combined O_2, 22·8 vols. %. 70 cc. blood in the large saturator.

21°.

	mm. Hg CO_2	Vols. % CO_2 total
First	29·0	47·9 after 30 mins.
Then	740	185·5 „ 30 „
„	„	184·3 „ 65 „
Afterwards	29·0	49·8 „ 30 „
„	„	47·8 „ 60 „
„	„	47·7 „ 120 „

In Table LVIII and in Figs. 20 and 21 experiments and algebraic smoothing will be found referring to different sera. These curves also appear to be straight lines although a number of points at high CO_2 tensions fall a little below the curve. It should however be noted that deviations from a straight line course are more difficult to detect than in the case of the blood curves on account of the relatively small value of x.

That serum and plasma really form a dissociable combination with CO_2 is raised beyond all doubt and has already been demonstrated by Setschenow [1879] and by Jaquet [1892], and later by Hasselbalch [1916, 2] and Hasselbalch and Warburg [1918] among others. It is also quite certain that varying

carbonic acid is combined as bicarbonate, as is shown by Loewy and Zuntz [1894, 1] and by Gürber's [1895, 2] old diffusion experiments. Gürber even claimed to have proved that CO_2 quantitatively was present as bicarbonate, a conclusion which is strongly supported by the determinations of $p\lambda_{(s)}$ in chapter VI of this work.

Table LVIII.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(s)}$ calculated	Vols. % CO_2 combined calculated	Difference
	Ox serum 38°. Hasselbalch and Warburg. I.			
41·3	54·4	7·42	54·1	+0·3
74·5	58·5	7·20	58·6	−0·1
140·5	62·2	6·95	63·7	−1·5
203·0	67·5	6·82	66·4	+1·1
271·5	68·9	6·71	68·6	+0·3
710·0	76·3	6·33	76·4	−0·1
	$x=20{\cdot}507\pm0{\cdot}2$. $y=206{\cdot}23\pm0{\cdot}2$. M = 1·0.			
	Ox serum 19° (serum B). Hasselbalch and Warburg. II.			
19·5	61·6	7·73	Unsuitable for calculation (see Fig. 20)	
79·8	69·6	7·17		
182·0	73·0	6·82		
378·3	73·7	6·52		
	Horse serum 38°. III.			
23·5	63·8	7·73	63·9	−0·1
24·6	63·4	7·71	64·1	−0·7
45·6	67·5	7·47	67·0	+0·5
104·6	71·8	7·14	71·0	+0·8
430·3	77·3	6·57	77·8	−0·5
	$x=12{\cdot}016\pm0{\cdot}2$. $y=156{\cdot}76\pm0{\cdot}2$. M = 0·7.			
	Horse serum 38°. IV.			
10·4	58·1	8·05	57·9	+0·2
35·2	63·3	7·56	63·6	−0·3
106·7	69·2	7·11	68·9	+0·3
130·7	69·7	7·03	69·8	−0·1
535·6	74·9	6·42	76·9	−2·0
	$x=11{\cdot}644\pm0{\cdot}1$. $y=151{\cdot}67\pm0{\cdot}1$. M = 0·3.			
	Ox serum 38°. V.			
22·7	62·6	7·74	64·2	−1·6
59·5	69·0	7·37	69·4	−0·4
103·7	74·7	7·16	72·4	+2·3
510·7	80·1	6·50	81·6	−1·5
	$x=14{\cdot}012\pm0{\cdot}6$. $y=172{\cdot}69\pm0{\cdot}6$. M = 2·3.			
	Human serum 38°. VI.			
15·2	57·2	7·88	58·7	−1·5
18·3	60·7	7·82	59·5	+1·2
84·5	68·7	7·21	68·2	+0·5
562·1	78·7	6·45	79·0	−0·3
	$x=14{\cdot}237\pm0{\cdot}3$. $y=170{\cdot}83\pm0{\cdot}3$. M = 1·4.			
	Horse serum 19°. VII.			
12·7	55·8	7·86	56·5	−0·7
18·7	58·2	7·71	57·9	+0·3
41·2	62·6	7·40	60·9	+1·7
119·6	64·4	6·95	65·2	−0·8
512·5	71·1	6·36	70·9	+0·2
	$x=9{\cdot}5901\pm0{\cdot}2$. $y=131{\cdot}87\pm0{\cdot}2$. M = 1·2.			

Table LVIII (*continued*)

Horse serum (19·5°) 20°. VIII.

mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'(e)}$ calculated	Vols. % CO_2 combined calculated	Difference
13·1	55·6	7·86	56·7	−1·1
34·1	61·1	7·48	60·5	+0·6
83·1	65·3	7·13	64·0	+1·3
573·7	71·4	6·32	72·2	−0·8

$x = 10{\cdot}025 \pm 0{\cdot}3$. $y = 135{\cdot}51 \pm 0{\cdot}3$. M = 1·4.

Ox serum 19°. IX.

16·0	58·5	7·78	59·3	−0·8
43·1	64·7	7·40	63·9	+0·8
118·6	69·4	6·99	68·9	+0·5
545·9	75·9	6·37	76·4	−0·5

$x = 12{\cdot}127 \pm 0{\cdot}2$. $y = 153{\cdot}65 \pm 0{\cdot}2$. M = 0·9.

Ox serum 21°. X.

16·1	61·8	7·81	61·4	+0·4
40·5	66·0	7·44	66·0	0·0
40·6	65·3	7·44	66·0	−0·7
70·2	68·5	7·22	68·8	−0·3
122·9	71·9	7·00	71·5	+0·4
595·0	75·4	6·33	79·8	−4·4

$x = 12{\cdot}412 \pm 0{\cdot}2$. $y = 158{\cdot}38 \pm 0{\cdot}2$. M = 0·7.

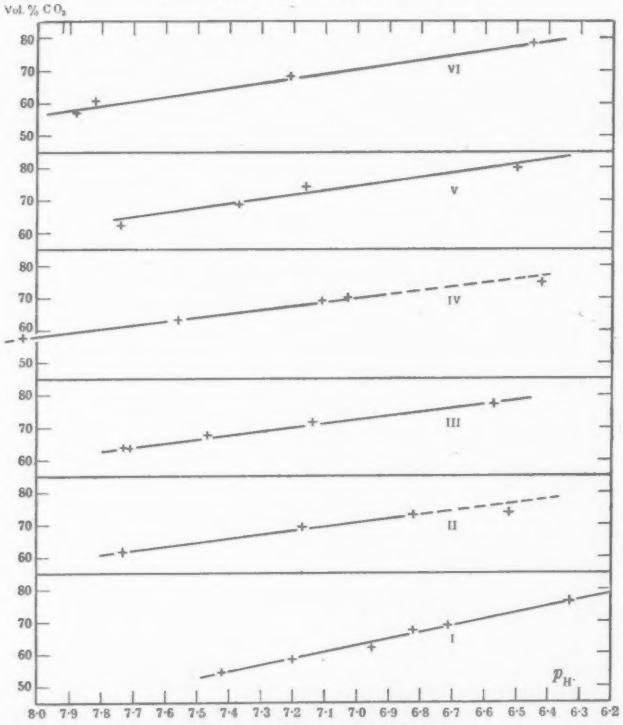

Fig. 20. The combination of CO_2 by sera.

In a paper on the neutrality of the blood W. M. Bayliss [1919] has recently advanced the opinion that the proteins of serum and plasma could not function as ampholytes at the reactions obtaining in my experiments and in the above mentioned papers. I do not agree with Bayliss on this point and as the subject is of prime importance for the problems we are discussing I shall attempt briefly to dispute his statements.

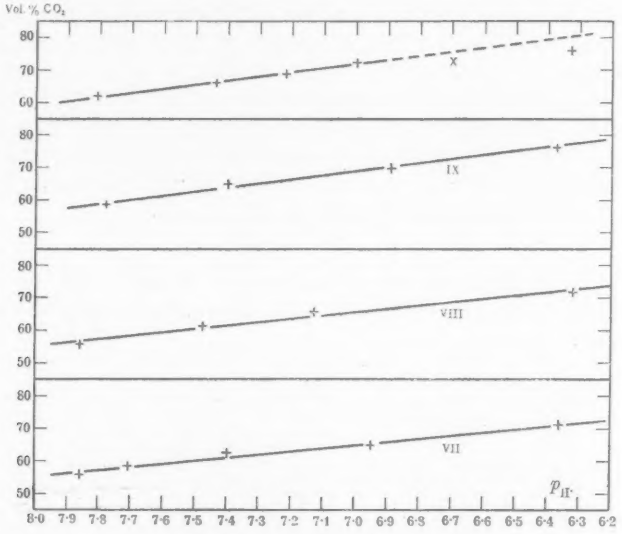

Fig. 21. The combination of CO_2 by sera.

Bayliss[1] asserts that serum does not combine with CO_2 in a reversible manner at the usual CO_2 tensions, supporting this by an experiment without doubt wrongly interpreted. It is obvious from the experiment that the variable combination—which Bayliss considers unimportant—is of the same order as that found by other authors. Bayliss further maintains that proteins do not act as buffers at reactions in the neighbourhood of the neutral point. This result is absolutely opposed to the finding of L. J. Henderson [1909–1910] and T. B. Robertson [1908–1910, 1912] with serum globulin and to the above mentioned experiments. The reason he has come to this erroneous conclusion must be that he has overrated the accuracy with which the reaction of plasma can be determined colorimetrically. In the same paper W. M. Bayliss has concluded by analogy that *haemoglobin* cannot act as a buffer. This analogy is however unjustifiable, quite apart from whether the experiments with plasma are correct or not, because the ampholytic character of the various

[1] Mukai (*J. Physiol.* 1921, **55**, 356) has quite recently, in Bayliss' laboratory, found that serum combines with variable amounts of CO_2 of a similar order to those found by earlier authors.

proteins is very different as is undoubtedly shown by the work of Robertson [1912] and his collaborators, and by the studies of Pauli [1920] and his co-workers.

In Table LIX and Fig. 22 the calculations from Campbell and Poulton's [1920] experiments with dialysed haemoglobin solution at 38° will be found. The corresponding curves are here also straight lines allowing for experimental error. As $2226C_I$ was 91·5 vols. % in the experiments the isoelectric point is 7·14. This is, as Campbell and Poulton [1920] themselves have remarked, considerably more alkaline than Michaelis and Takahashi [1910] found at room temperature the isoelectric point according to these observers being p_H· (Bjerrum) 6·79. Now it can be shown from a glance at the CO_2 combination curves—as Hasselbalch [1916, 2] has pointed out—that the isoelectric point for haemoglobin must be more acid at body temperature than at room temperature (the difference is about 0·30), so that the difference between Campbell and Poulton's and Michaelis' results is real[1].

Table LIX. Dialysed solution of haemoglobin (Haldane 52) with 0·041*n* $NaHCO_3$, 38°; $p\lambda_{(m)} = 6{\cdot}23$. Calculated after J. M. H. Campbell and E. P. Poulton.

mm. Hg CO_2	Vols. % CO_2 combined	p_H· calculated	Vols. % CO_2 combined calculated	Difference
4·8	52·3	8·40	36·2	+16·1
4·9	52·1	8·39	36·6	+15·5
11·0	57·5	8·08	50·3	+ 7·2
21·9	63·3	7·83	61·2	+ 2·1
40·7	72·9	7·62	70·4	+ 2·5
43·6	76·5	7·61	70·9	+ 5·6
50·7	75·5	7·54	70·0	+ 5·5
52·5	70·2	7·47	77·0	− 6·8
78·1	74·1	7·34	82·8	− 8·7
85·5	79·8	7·34	82·8	− 3·0
90·0	77·1	7·30	84·5	− 7·4
110·5	92·3	7·29	85·0	+ 7·3
119·8	86·3	7·22	88·0	− 1·7
151·4	93·5	7·16	90·7	+ 2·8
221·9	99·1	7·02	96·8	+ 2·3
226·7	97·8	7·00	97·7	+ 0·1
310·4	101·9	6·88	103·0	− 1·1
338·0	107·0	6·87	103·4	+ 3·6
435·3	110·0	6·77	107·8	+ 2·2
596·4	112·2	6·64	113·5	− 1·3

$x = 43{\cdot}959 \pm 0{\cdot}2$. $y = 405{\cdot}41 \pm 0{\cdot}2$. $M = 4{\cdot}5$.

Some experiments I have performed give an indication of the position of the isoelectric point. According to Hardy the agglutination optimum of

[1] T. R. Parsons and Winifred Parsons (*Biochem. Zeitschr.* 1921, **126**, 108) have recently pointed out that Campbell and Poulton's haemoglobin solutions must have contained inorganic salts because when they incinerated the haemoglobin they found more ash than could have come from the iron alone. Calculating the amount of HCO'_3 which would be balanced by metallic ions on the assumption the excess of ash was K_2CO_3 we get 30 vols. % and the isoelectric point is therefore about 6·5, which agrees with Michaelis' determination when we take the difference of temperature into account.

a colloid is at the isoelectric point. The crystallising out of haemoglobin at high CO_2 tensions (after saponin haemolysis) mentioned in chapter IV never began at reactions more alkaline than p_H· 7·10 at room temperature under the conditions of the experiment, but the ease with which crystallisation was set going increased until p_H· 6·80 was reached which supports Michaelis' result. Should further experiments confirm the difference between the isoelectric point estimated from the combined CO_2 and from the other method, it will indicate that some CO_2 is adsorbed or combined in a complex manner with the haemoglobin.

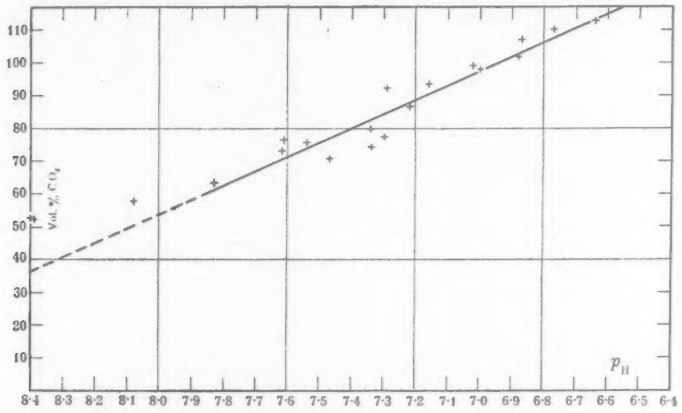

Fig. 22. CO_2 combined by dialysed haemoglobin (with the addition of 0·041n $NaHCO_3$). (J. M. H. C. & E. P. P.)

With the help of the relations developed in this chapter, which found their simplest expression in (173) and (176), it will be possible to investigate whether all the reversibly combined CO_2 is present as bicarbonate. Before reporting the experiments I have made in this connection I would draw attention to the fact that an error in $p\lambda_{(s)}$ or $p\lambda_{(m)}$ or in the correction taken from Fig. 10 is without significance here, because it will influence the estimation of the hydrogen ion exponent in the original and in the curve displaced by the addition of alkali to the same extent and thus the relative positions of the curves will not be altered.

The experiments were carried out as follows: blood or saponin haemolysate ($\frac{1}{2}$ % saponin) which was slightly concentrated by pipetting off serum was sloped in a 100 cc. measuring flask so that it filled about half of it. With a pipette either 5 cc. $n/2$ NaCl solution or 5 cc. $n/2$ $NaHCO_3$ solution was then added during shaking, the titre of which was determined beforehand by the exhaust pump. Two drops of octyl alcohol were next added to prevent frothing after which the flask was filled up with blood (or haemolysate).

With the mixtures thus prepared CO_2 combination curves were constructed with the help of the large saturator. The calculations were made as before, and the results are given in Table LX and in Figs. 23, 24 and 25.

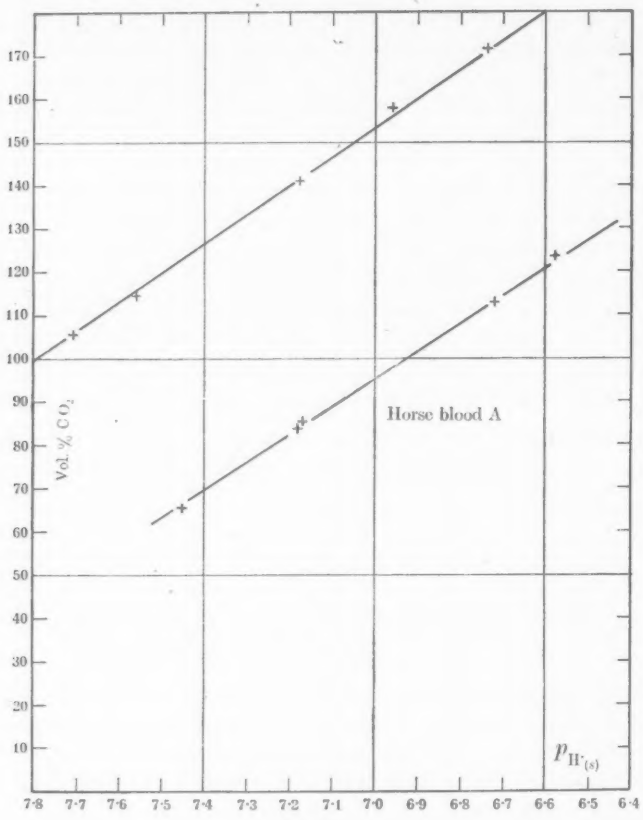

Fig. 23.

Let us first compare the constant x.

		Lower curve	Upper curve	Difference
Horse blood A		66·9±0·3	69·2±0·3	2·3±0·4
Horse blood haemolysate	B	87·0±0·6	89·2±0·5	2·2±0·7
Ox " "	C	57·9±1·1	57·8±0·9	−0·1±1·4
Horse " "	D	81·3±0·1	83·3±0·3	2·0±0·3
" " "	E	64·0±0·1	70·3±0·3	6·3±0·3

Table LX.

Defibrinated horse blood A. Curve I. $\Psi = 11$.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'(e)}$ calculated	Vols. % CO_2 combined calculated	Difference vols. %
17	43·5	147·0	65·6	26·5	7·45	65·6	0·0
,,	99·8	135·5	83·8	26·4	7·18	83·7	+ 0·1
,,	104·6	—	85·4	26·4	7·17	84·3	+ 1·1
,,	358·8	—	112·7	—	6·72	114·4	− 1·7
,,	537·8	—	123·5	—	6·59	123·1	+ 0·4
			Mean	26·4			

$x = 66{\cdot}883 \pm 0{\cdot}3$. $y = 563{\cdot}87 \pm 0{\cdot}3$. M = 1·2. Theoretical distance 57·0.

Curve II.

17	40·4	147·6	105·6	26·9	7·71	104·9	+ 0·7
,,	59·9	143·7	114·5	26·5	7·56	115·3	− 0·8
,,	168·0	120·5	140·9	26·4	7·18	141·6	− 0·7
,,	303·8	—	158·1	—	6·96	156·8	+ 1·3
,,	522·4	—	171·5	—	6·74	172·0	− 0·5
			Mean	26·6			

$x = 69{\cdot}172 \pm 0{\cdot}3$. $y = 638{\cdot}24 \pm 0{\cdot}3$. M = 1·1.

Saponin haemolysed horse blood B. Curve I. $\Psi = 11$.

18	14·4	—	33·6	26·2	7·60	31·3	+ 2·3
,,	45·2	—	54·2	25·9	7·31	56·5	− 2·3
,,	84·8	—	69·3	—	7·15	70·4	− 1·1
,,	359·3	—	107·4	—	6·71	108·7	− 1·3
,,	536·8	—	121·4	—	6·59	119·2	+ 2·2
,,	137·4	—	75·0	—	6·97	86·1	− 11·1[1]
,,	359·3	—	92·6	—	6·64	114·8	− 22·2[1]

[1] Crystallisation.

$x = 87{\cdot}033 \pm 0{\cdot}6$. $y = 692{\cdot}70 \pm 0{\cdot}6$. M = 2·5. Theoretical distance 53·0.

Curve II.

18	20·3	—	69·4	—	7·77	68·3	+ 1·1
,,	45·6	—	89·0	—	7·52	90·6	− 1·6
,,	70·6	—	102·5	—	7·40	101·3	+ 1·2
,,	141·6	—	120·8	—	7·16	122·7	− 1·9
,,	360·3	—	150·7	—	6·86	149·5	+ 1·2

$x = 89{\cdot}176 \pm 0{\cdot}5$. $y = 761{\cdot}21 \pm 0{\cdot}5$. M = 1·8.

Saponin haemolysed ox blood C. Curve I. $\Psi = 8$.

19	19·4	—	38·7	20·6	7·54	37·7	+ 1·0
,,	60·2	—	56·7	—	7·21	56·8	− 0·1
,,	394·1	—	93·6	—	6·62	91·0	+ 2·6
,,	166·9	—	73·6	—	6·86	77·1	− 3·5

$x = 57{\cdot}899 \pm 1{\cdot}1$. $y = 474{\cdot}27 \pm 1{\cdot}1$. M = 3·2. Theoretical distance 54·5.

Curve II.

19	44·4	—	91·1	20·3	7·55	91·6	− 0·5
,,	398·5	—	135·4	—	6·77	136·6	− 1·2
,,	164·9	—	120·1	—	7·10	117·6	+ 2·5
,,	82·3	—	102·9	—	7·34	103·7	− 0·8

$x = 57{\cdot}772 \pm 0{\cdot}9$. $y = 527{\cdot}75 \pm 0{\cdot}9$. M = 2·1.

Saponin haemolysed horse blood D. Curve I. $\Psi = 11$.

18	18·3	133·9	36·7	25·0	7·53	36·4	+ 0·3
,,	18·3	133·9	36·5	24·4	7·53	36·4	+ 0·1
17	52·9	—	57·8	—	7·26	58·3	− 0·5
,,	91·9	—	70·3	—	7·11	70·5	− 0·2
18	531·8	—	114·6	—	6·57	114·4	+ 0·2
17	153·4	—	81·5	—	6·95	83·5	− 2·0[1]
,,	348·0	—	97·4	—	6·67	106·3	− 8·9[1]

[1] Crystallisation.

$x = 81{\cdot}260 \pm 0{\cdot}1$. $y = 648{\cdot}25 \pm 0{\cdot}1$. M = 0·5. Theoretical distance 54·5.

Table LX (*continued*)

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'(\omega)}$ calculated	Vols. % CO_2 combined calculated	Difference Vols. %
			Curve II.				
18	532·4	—	157·0	—	6·70	157·3	− 0·3
17	16·8	—	66·9	—	7·82	64·0	+ 2·9
,,	52·6	—	91·9	—	7·47	93·2	− 1·3
,,	72·8	—	100·0	—	7·36	102·3	− 2·3
,,	147·5	—	120·4	—	7·14	120·7	− 0·3
,,	515·9	—	158·3	—	6·71	156·5	+ 1·8
,,	320·0	—	141·8	—	6·87	143·2	− 1·4[1]

[1] Crystallisation.

$x = 83{\cdot}322 \pm 0{\cdot}3$. $y = 715{\cdot}57 \pm 0{\cdot}3$. M = 1·6.

Saponin haemolysed horse blood E. Curve I. $\Psi = 11$.

18	19·8	—	41·7	25·7	7·55	41·7	0·0
,,	61·7	—	61·6	—	7·23	62·2	− 0·6
17	80·0	—	68·0	—	7·15	67·3	+ 0·7
,,	155·6	—	80·9	—	6·94	80·8	+ 0·1
,,	342·3	—	97·1	—	6·68	97·4	− 0·3
,,	545·9	—	107·8	—	6·52	107·7	+ 0·1

$x = 64{\cdot}052 \pm 0{\cdot}1$. $y = 525{\cdot}29 \pm 0{\cdot}1$. M = 0·5. Theoretical distance 54·5.

Curve II.

18	22·1	—	78·6	26·0	7·78	77·1	+ 1·5
,,	101·9	—	111·3	—	7·27	113·0	− 1·7
,,	185·9	—	126·1	—	7·06	127·8	− 1·7
17	301·3	—	140·0	—	6·89	139·8	+ 0·2
,,	514·8	—	153·3	—	6·70	153·1	+ 0·2
,,	512·3	—	154·7	26·1	6·70	153·1	+ 1·6

$x = 70{\cdot}335 \pm 0{\cdot}3$. $y = 624{\cdot}37 \pm 0{\cdot}3$. M = 1·6.

Apart from ox blood haemolysate C, which appears to be subject to a far greater fortuitous error than the other members of the series, the upper curves all exhibit a rather steeper ascent than the lower ones. Although we should be very cautious, as previously explained, in drawing definite conclusions from the size of the mean error about an actual discrepancy between the constants it is extremely probable the upper curve really has a somewhat steeper course than the lower. In accordance with this the equation for the combination of CO_2 in the blood and haemolysate should not be

$$(173) \quad B = (C_I - C_V f(a_{H'}))\, 2226,$$

but

$$(176) \quad B = (C_I - C_V f(a_{H'}) - f_1(P_{CO_2}))\, 2226,$$

and therefore some CO_2 should be combined in blood in some other form than bicarbonate. There is however an alternative explanation which seems to me more probable. As will be remembered it was explained on p. 280 that the difference between the curves at no place could be less than 2226*b*, which is the value entered in the tables as the "theoretical distance." We will now investigate how far this is correct.

		B		Difference		
		At $p_{H'}$ 7·50	At $p_{H'}$ 6·70	At $p_{H'}$ 7·50	At $p_{H'}$ 6·70	Theoretical distance
Horse blood A.	Upper curve	119·5	174·8	56·5	60·5	57·0
	Lower „	63·0	114·3			
„ „ haemolysate B.	Upper „	92·4	163·8	52·4	55·2	53·0
	Lower „	40·0	108·6			
Ox „ „ C.	Upper „	94·3	140·7	54·3	54·3	54·5
	Lower „	40·0	86·4			
Horse „ „ D.	Upper „	90·7	157·3	51·9	53·5	54·5
	Lower „	38·8	103·8			
„ „ „ E.	Upper „	96·9	153·1	52·0	57·0	54·5
	Lower „	44·9	96·1			

There seems therefore to be an inclination to obtain too small differences in the most alkaline reactions. In the case of the haemolysates D and E this seems to exceed the experimental error as the latter may be estimated as below 2 vols. % of the difference between the curves, determined by five points or more. (The standard deviation for all the determinations with the

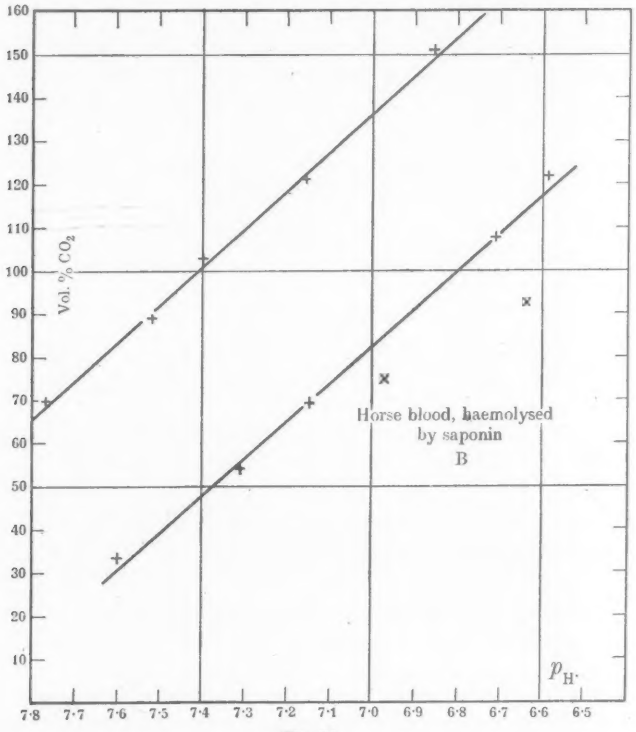

Fig. 24.

exception of the horse blood haemolysate D is 1·2 vols. %.) A possible explanation of the phenomenon is that on the addition of bicarbonate the blood was very little changed so that before the bicarbonate was completely mixed with the blood a slight decomposition of the proteins took place with the formation of new acid radicles.

The result of these experiments is therefore that it is probable that CO_2 is combined in the blood only as carbonic acid, that is to say as bicarbonate ion, but that the possibility cannot be entirely excluded that small quantities may be combined in other ways.

In the haemolysates B and D haemoglobin crystallised out in the acid reactions. It will be noticed that the combination of CO_2 decreases when crystallisation takes place but it must be remembered we cannot be sure of getting a homogeneous sample of the whole system when the crystal phase is in process of formation. Setschenow [1879] has observed a similar decrease of the combined CO_2 of horse blood when the haemoglobin crystallised out.

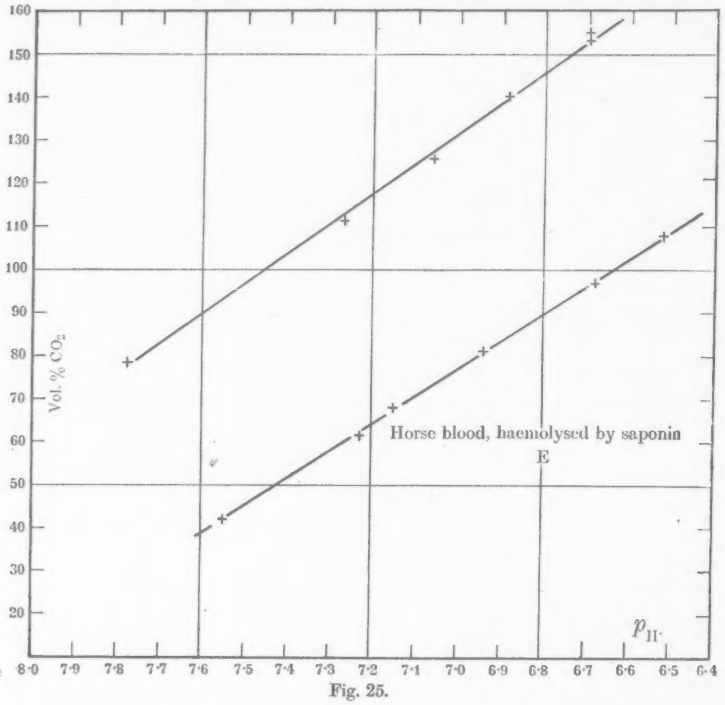

Fig. 25.

In conclusion I will report some experiments carried out in a similar manner with histidine hydrochloride with which Prof. Henriques kindly supplied me, which make it probable the CO_2 combination curve of histidine

can be shifted in a $p_{H'}$–vols. % CO_2 diagram in a similar way to that of blood. The preparation was impure as it only contained 17·1 % N_2 while the theoretical amount for histidine mono-hydrochloride is 26·5 % and for histidine di-hydrochloride 21·5 %. A 5 % solution was 0·439*n* as regards chlorine which corresponds to a mixture of mono- and di-hydrochloride. It can however be concluded from the course of the CO_2 combination curve that chloride must also have been present in some other form than histidine hydrochloride. The solutions were prepared in such a way that for the lower curve a 5 % solution of histidine hydrochloride was mixed with an equal part of a sodium bicarbonate solution which combined with 995·9 vols. % just before each experiment, while for the upper curve a bicarbonate solution which combined with 1106·5 vols. % was used. The theoretical distance between the curves should be 55·3 vols. %. The experiments will be found in Table LXI and in Fig. 26. It will be observed the curves are very nearly parallel with an intervening distance of 53·5 to 57·5 vols. % which may be regarded as sufficiently accurate for curves of this type. It should be remarked however that the distance between the curves increases a little with increasing apparent hydrogen ion activity and that the distance in the most alkaline reactions is about 2 vols. % less than the theoretically smallest possible distance so that there must either be small experimental errors or the theory proposed must be incomplete in one way or another.

Table LXI. 2·5 % histidine hydrochloride $p_{K'_I} = 6{\cdot}300$ at 18° and 6·305 at 17°. $Y = 8$.

	Curve I.				Curve II.		
Temp.	mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'}$ calculated	Temp.	mm. Hg CO_2	Vols. % CO_2 combined	$p_{H'}$ calculated
17°	22·5	35·7	7·41	18°	23·5	76·1	7·72
,,	40·5	44·3	7·24	,,	48·8	86·8	7·46
,,	83·4	57·7	7·05	,,	113·2	101·6	7·17
,,	188·5	78·7	6·83	,,	207·7	119·0	6·97
,,	392·3	103·0	6·63	,,	390·4	141·7	6·77
,,	731·0	127·5	6·45	,,	730·0	167·5	6·50

Theoretical distance 55·3 vols. %.

In the above discussion an assumption has been made which is not really tenable, namely that a complex combination between the CO_2 and proteins or histidine does not drive CO_2 out of the bicarbonate. If such a combination, for example a carbamino-acid, is in itself an acid it will in virtue of its cations be able to drive out CO_2. Equation (176) should therefore—if this possibility be allowed—have the form

$$B = (C_I - C_V f(a_{H'}) - f_1(P_{CO_2}) - f_2(P_{CO_2}, a_{H'}))\,2226,$$

and the above experiment would be explained without the assumption of an experimental error. Further experiment can alone determine whether this explanation accords with fact but the above experiment proves at any rate that only very small amounts of carbamino-acid can be formed at the reactions investigated.

As the final result of the investigations reported in chapters VIII, IX, X and XI it is clear that the CO_2 of the blood is exclusively or almost exclusively combined as bicarbonate and that the variable amount combined is due to the presence in the blood of electrolytes the dissociation of which varies with the reaction. Such electrolytes are chiefly proteins. On account of the relatively small concentration of the serum proteins their effect will be subordinate to that of the proteins of the blood corpuscles. Haemoglobin is the most active of the blood corpuscle proteins judging from experiments of Setschenow [1879], Bohr [1905], Hasselbalch [1916, 2] and Campbell and Poulton [1920]. It is impossible to estimate the activity of haemoglobin in this respect but it is not improbable that the other proteins and lecithin (Setschenow) are also active. To a limited extent the phosphates will also function in a similar manner.

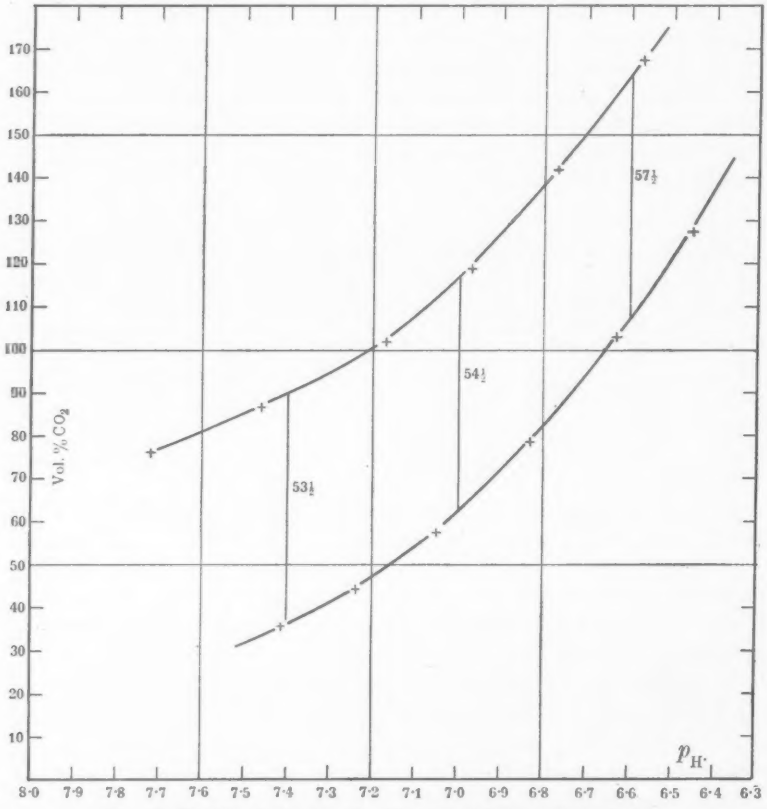

Fig. 26. Histidine hydrochloride. Theoretical distance 55·3 vols. %.

RÉSUMÉ.

I. The theory of the combination of CO_2 in a solution which contains both electrolytes the dissociation of which does and does not vary with the reaction is elaborated.

II. This theory has been tested on phosphate solutions and found to be satisfactory.

III. An empirical relation on a rational basis has been proposed for the combination of CO_2 in serum, haemoglobin solutions and blood.

IV. It has been shown it is only possible for a trifling amount of CO_2 to be adsorbed or bound in a complex manner (as carbamino-acid) in blood or haemolysate.

V. A similar proof has been produced for the combination of CO_2 in a histidine solution.

CHAPTER XII

THE FACTORS WHICH DETERMINE THE PARTITION OF PERMEATING IONS BETWEEN THE BLOOD CORPUSCLES AND THE SERUM, THE VOLUME OF THE BLOOD CORPUSCLES DEPENDENT UPON THE REACTION, AS WELL AS THE POTENTIAL ON THEIR SURFACES

As repeatedly stated A. Schmidt [1867] and N. Zuntz [1867] discovered independently of one another that the amount of alkali in serum increased when the blood was treated with high tensions of CO_2. Zuntz explained this phenomenon by assuming that the effect of CO_2 was to split off sodium from the sodium-protein compounds of the blood cells and that some of the sodium diffused out of the blood cells in the form of sodium bicarbonate until the sodium bicarbonate concentration was the same in blood cells and serum. When we remember the state of physical, and especially physiological, chemistry at that time the absolute genius displayed in Zuntz's hypothesis excites the greatest admiration.

The converse of this bicarbonate diffusion was discovered in 1874 without its importance in this connection being noticed. Hermann Nasse[1], who at that time was an old investigator and who had gained a considerable reputation in haematology, discovered in 1874 that the chlorine in serum decreased when blood was treated with high CO_2 tensions. At the same time he found that the blood cells swelled under CO_2 treatment, taking up water from the serum. Although he again reported his discovery in *Pflüger's Archiv* in 1878 his name has quite disappeared from textbooks on physico-chemical biology because the honour of discovering the phenomena in question was falsely ascribed to Hamburger [1892, 1902] and v. Limbeck [1894]. In the literature

[1] Do not confuse with the son O. Nasse, the discoverer of isotonic salt solution, or with the father, the celebrated clinician of Bonn, Christian Friedrich Nasse.

dealing especially with ionic equilibrium in the blood I have only found H. Nasse mentioned by Gürber [1895, 1].

When it was decisively shown by the work of Gryns, Hamburger [1902], Hedin [1915], Overton, Gürber [1895], Koeppe [1897] and many others about the beginning of the century that the membrane of the blood cells was impermeable for cations—Arrhenius' ionic theory was at that time generally accepted by physiologists—it became necessary to seek a new explanation of the diffusion of ions between blood cells and serum. From excellent but wrongly interpreted experiments Gürber [1895] formed a theory that CO_2 split up NaCl with the formation of free HCl and sodium bicarbonate in serum and that the HCl subsequently diffused into the blood cells.

Koeppe [1897] advanced the theory that by CO_2 treatment monocarbonate ions are formed in the blood cells and that these are exchanged with chlorine ions from the serum—a theory which is only a special application of the principle enunciated by W. Ostwald in 1890 for ionic diffusion through a semi-permeable membrane. In his handbook Hamburger [1902] has thoroughly dealt with these theories but considers it is impossible to judge which is right. E. Petry [1903] has partly identified himself with Gürber's theory. But Gürber's theory is undoubtedly fallacious because firstly carbonic acid could not split up an appreciable amount of NaCl, even if the latter was not completely dissociated, and secondly, the same CO_2 tension must be present in the blood cells and serum, so that the CO_2 must have the same effect on the NaCl on both sides of the membrane. Koeppe's theory was falsified by the fact that he assumed monocarbonate ions could be formed in appreciable quantities by CO_2 treatment, but on account of the hydrogen ion activity this is impossible as shown by L. Meyer [1857], Setschenow [1879], Bohr [1905] and others. But if we substitute bicarbonate ions for monocarbonate ions Ostwald and Koeppe's theory is sound. Koeppe was not clear why more carbonate ions should be formed in the blood cells than in serum when the blood is treated with high CO_2 tensions but we can fall back upon Zuntz's [1882] old theory for the explanation, and it is of little importance in this connection whether we imagine the sodium to be originally combined in a complex manner with the proteins of the blood cells or whether we believe it to be present as ions partially balanced by electrolytes the dissociation of which varies with the reaction.

Loewy and Zuntz [1894] have imitated the ionic exchange process by enclosing serum or blood cell fluid in a parchment dialysing bag according to Kühne and dialysing against salt solutions, and they have shown that by treating the inner liquid with CO_2 alkali bicarbonate passed into the outer liquid. Loewy and Zuntz knew that alkali bicarbonate was liberated in this way both in the plasma and in the blood cells, but that by far the greatest amount appeared in the blood cells, and they have drawn attention to the connection between this phenomenon and the great capacity of the blood cells for combining with acid. Gürber [1895, 2] and Spiro and Henderson

[1909] have similarly imitated the exchange of ions with protein and salt solutions separated by parchment membranes with the same results. Spiro and Henderson have put the question very concisely by combining Koeppe's hypothesis (with the revision mentioned) with Loewy and Zuntz's views concerning the different combining properties of blood cells and serum. Hasselbalch and Warburg [1918], without at the time being cognisant of Spiro and Henderson's work, advanced a similar interpretation of the phenomenon, but taking into account the modern view of proteins as ampholytes, which however is not of great moment for the theory. Loewy and Zuntz, Gürber, Rona and György [1913, 2] have made it very probable by dialysis experiments that the greater part of the combined CO_2 in serum is present as bicarbonate, but the explanation of the experiments involves many difficulties. Gürber, Loewy and Zuntz, C. Lehmann [1894] and Petry have shown that the alkali metals do not diffuse on treating the blood with CO_2; the small differences found in the experiments are undoubtedly partly due to slight inaccuracies in the analyses and partly to the fact that the authors, with the exception of Petry, have disregarded volume changes of the blood cells. Hamburger and Bubanovic [1910] alone found a wandering of cations in some briefly reported experiments but this result is in opposition to what is known otherwise of the permeability of the membrane of the blood cells. Reference should also be made to the handbooks and to Ege's important work of 1920.

L. J. Henderson [1920] has quite recently pointed out that O_2 and CO_2 combinations in blood, and Cl and bicarbonate in serum, are so related that we can conclude from the phase rule that there is a common cause for their mutual equilibria, but he did not explain it in detail. A question of considerable interest with regard to the considerations which are to follow is, how many inorganic cations there are in blood cells and serum, but the question cannot be solved at present as the published analyses do not agree with one another. The distribution of inorganic cations between the external and internal phases of the blood cells is unknown but we shall not be far wrong in assuming the concentration in the external phase to be about 0·15*m*. We can briefly express the Zuntz theory of the distribution of ions between blood cells and serum, taking into account the advance since Zuntz propounded his theory and remembering the work of Koeppe, Zuntz and Loewy, Rona and György, and Spiro and Henderson, as follows.

The blood cells are surrounded by a membrane impermeable to proteins and cations. Both blood cells and serum (plasma) contain electrolytes the dissociation of which varies with the reaction, but in the neighbourhood of the neutral point the content of the blood cells in these substances is greatly in the ascendant. Thus more bicarbonate ions are formed in the blood cells than in the serum (and fewer hydrogen ions in the blood cells than in the serum), when the blood is brought into equilibrium with an atmosphere in which the CO_2 tension is higher than that with which the blood was originally in equilibrium. As the equilibrium is disturbed in this way bicarbonate ions

from the blood cells are exchanged with other anions (chiefly chlorine ions) from the serum till equilibrium is again established—until the apparent bicarbonate ion activity in the blood cells and serum is *the same*. It is of no importance whether the bicarbonate ions are balanced by inorganic or by protein cations. The above form of Zuntz's theory is subject to a considerable defect which was foreign to the original theory. While Zuntz thought a part of the alkali of the blood cells was combined undissociated to the blood cell proteins and that these compounds were split up on treatment with CO_2 allowing sodium bicarbonate to diffuse from the blood cells into the serum until the concentration was the same in both, we must assume, since Gryns, Koeppe, Hamburger [1902], Hedin [1915], Gürber [1895 1, 2] and others have demonstrated the impermeability of the membrane of the blood cells to cations, that bicarbonate ions are exchanged for other anions (chiefly Cl′) until the concentration is the same in blood cells and serum. This opens up a new problem which has never been adequately discussed. If there was equilibrium before the CO_2 treatment of the blood between the chlorine ions in the blood cells and serum it requires explaining how it is possible there can also be an equilibrium after chlorine ions have wandered from the serum into the blood cells. In the Zuntz theory as modified by Loewy and Zuntz, Koeppe, Spiro and Henderson, and Hasselbalch and Warburg the assumption has however been made that the bicarbonate ion concentration or as it ought to be expressed, the apparent bicarbonate ion activity, is the same in the blood cells and the serum independent of the reaction (and CO_2 tension). If on the other hand we assumed that the exchange of ions only proceeded until the relative decrease in tension of each kind of ion between blood cells and serum was the same we should have a rational explanation.

It will now be shown thermodynamically with the help of Donnan's [1911] distribution law that this explanation is a necessary consequence provided the assumptions relating to the permeability of the membrane of the blood cells and the electrolytes the dissociation of which varies with the reaction are correct.

As early as 1890 W. Ostwald showed that diffusible ions must distribute themselves in a characteristic manner between two solutions separated by a membrane when ions were present which could not pass through it. Ostwald's demonstration of this fact has however not been very fruitful to physiology and its application to the problem of the unequal distribution of ions referred to above was on the whole very difficult to grasp until Donnan published his work. As I fully share Donnan's conviction that a knowledge of his distribution law will be of extreme importance for the appreciation of ionic equilibrium in the organism and a number of other physiological problems, *e.g.* in muscle physiology, I will shortly review the fundamental facts of the theory. A complete exposition is given in W. C. McC. Lewis' *A System of Physical Chemistry* [1916] as well as in Donnan's own paper, to which the reader is referred.

Let us imagine two solutions separated by a membrane which is impermeable to an anion R', while the ions $Na^{\cdot}$ and Cl' diffuse freely. There are originally only $Na^{\cdot}$ and R' in the one solution and only $Na^{\cdot}$ and Cl' in the other according to the scheme

$Na^{\cdot}$	$Na^{\cdot}$
R'	Cl'
(1)	(2)

.....(183)

When equilibrium is established $Na^{\cdot}$ and Cl' have diffused from (2) to (1) so that we now have

R'	$Na^{\cdot}$
$Na^{\cdot}$	
Cl'	Cl'
(1)	(2)

.....(184)

Diffusion goes on until no work can be done by the simultaneous isothermous transference of a differential mol of $Na^{\cdot}$ and Cl', so that we can obtain no energy by simultaneously carrying out the two isothermous differential processes

$$\delta n \text{ mol } Na^{\cdot}\,(2) \rightarrow (1),$$

$$\delta n \text{ mol } Cl'\,(2) \rightarrow (1).$$

We therefore have the equilibrium equation

$$\delta n \, R \, T \log_e \frac{C_{Na^{\cdot}\,(2)}}{C_{Na^{\cdot}\,(1)}} + \delta n \, R \, T \log_e \frac{C_{Cl'\,(2)}}{C_{Cl'\,(1)}} = 0. \quad \text{.....(185)}$$

Therefore

$$\frac{C_{Na^{\cdot}\,(1)}}{C_{Na^{\cdot}\,(2)}} = \frac{C_{Cl'\,(2)}}{C_{Cl'\,(1)}} \quad \text{.....(186)}$$

on the assumption the solutions are ideal.

Similarly for other ions in general we get

$$\frac{C_{Na^{\cdot}(1)}}{C_{Na^{\cdot}(2)}} = \frac{C_{M^{\cdot}{}_{I}\,(1)}}{C_{M^{\cdot}{}_{I}\,(2)}} = \frac{C_{A'_{I}\,(2)}}{C_{A'_{I}\,(1)}} = \sqrt[2]{\frac{C_{M^{\cdot\cdot}{}_{II}\,(1)}}{C_{M^{\cdot\cdot}{}_{II}\,(2)}}} = \sqrt[3]{\frac{C_{M^{\cdot\cdot\cdot}{}_{III}\,(1)}}{C_{M^{\cdot\cdot\cdot}{}_{III}\,(2)}}} = \sqrt[2]{\frac{C_{A''_{II}\,(2)}}{C_{A''_{II}\,(1)}}} \text{ etc.,}$$

.....(187)

where $M^{\cdot}{}_{I}$ is any monovalent cation and A'_{I} any monovalent anion and so on.

Donnan gives the following example: two solutions in equilibrium have originally the constitution

$Na^{\cdot}$	R'	$Na^{\cdot}$	Cl'
C_1	C_1	C_2	C_2
(1)		(2)	

.....(188)

where C_1 and C_2 are the molar ion concentrations in the solutions. Assuming complete activity and equal volumes of the solutions on either side of the membrane during the whole process, we get, when equilibrium is reached,

$Na^{\cdot}$	R'	Cl'	$Na^{\cdot}$	Cl'
$C_1 + x$	C_1	x	$C_2 - x$	$C_2 - x$
(1)			(2)	

.....(189)

From this it follows that $\frac{x}{C_2}\,100$ is the quantity of NaCl which diffuses from

(2) to (1) expressed as a percentage of the original amount of NaCl in (2), and $\frac{C_2-x}{x}$ is the distribution ratio of NaCl between (2) and (1) corresponding to the equilibrium.

The equation in this case is

$$x = \frac{C_2^2}{C_1+2C_2}, \quad \text{.....(I)}$$

from which

$$\frac{x}{C_2} = \frac{C_2}{C_1+2C_2}, \quad \text{.....(II)}$$

$$\frac{C_2-x}{x} = \frac{C_1+C_2}{C_2}. \quad \text{.....(III)}$$

If C_2 is small compared with C_1 we can put

$$\frac{x}{C_2} = \frac{C_2}{C_1}, \quad \text{.....(IV)}$$

and

$$\frac{C_2-x}{x} = \frac{C_1}{C_2}. \quad \text{.....(V)}$$

If we take for example $C_2 = \frac{1}{100} C_1$, then $\frac{x}{C_2} = \frac{1}{100}$, that is to say only $\frac{1}{100}$th of the original amount of NaCl diffuses from (2) to (1). If on the other hand C_1 is small compared with C_2

$$\frac{x}{C_2} = \frac{1}{2}, \quad \text{.....(VI)}$$

and

$$\frac{C_2-x}{x} = 1.$$

In the following table Donnan gives some calculations made by equations (I), (II) and (III).

Table LXII.

Original concentration of Na R in (1)	Original concentration of NaCl in (2)	Original ratio of Na R to NaCl	% NaCl diffused from (2) to (1)	Distribution ratio of NaCl between (2) and (1)
C_1	C_2	$\frac{C_1}{C_2}$	$\frac{x}{C_2}100$	$\frac{C_2-x}{x}$
0·01	1	0·01	49·7	1·01
0·1	1	0·1	47·6	1·1
1	1	1	33	2
1	0·1	10	3	11
1	0·01	100	1	101

It is clearly seen from the equations and tables that the ions for which the membrane is freely permeable will become very unevenly distributed between the two solutions if the concentration (activity) of the ions for which the membrane is impermeable is large in comparison with the former.

In Fig. 27 we have the conception of Donnan in schematic form. The figure shows the cross-section of a cylinder in which a piston can move. The piston represents the semi-permeable membrane. Solution (1) is on the left of the piston, solution (2) on the right. The piston is situated in the middle of the cylinder and is fixed there, so that the volumes of the two solutions are equal. The ions for which the piston is impermeable will attempt to force the piston to the right (as they are only present in solution (1)), and since the ions of opposite charge (in this case $Na^{\cdot}$) are held back in the same amount

by the electrostatic force, the osmotic pressure (the pressure on a square centimetre of the piston) will be

$$P_0 = 2C_1 \mathrm{R\,T}, \quad \text{.....(VII)}$$

provided that (2) contains only pure water. But as in the present example (2) also contains $Na^{\cdot}$ and Cl', and as these ions are unevenly distributed in (1) and (2), the sodium chloride will exert a counter-pressure on the piston when equilibrium is established which will be

$$P = 2\,(C_2 - x)\,\mathrm{R\,T} - 2x\mathrm{R\,T} = 2\,(C_2 - 2x)\,\mathrm{R\,T}. \quad \text{.....(VIII)}$$

The force per unit surface which will attempt to drive the piston to the right when equilibrium is established will then be

$$P_1 = P_0 - P = 2\mathrm{R\,T}\,(C_1 - C_2 + 2x). \quad \text{.....(IX)}$$

Therefore combining with (I) $\dfrac{P_1}{P_0} = \dfrac{C_1 + C_2}{C_1 + 2C_2}$, ...(X)

If C_1 is small compared with C_2, $P_1 = \frac{1}{2}P_0$; if C_2 is small compared with C_1, $P_1 = P_0$ as would be expected.

The following table (after Donnan) shows the relations:

$\frac{C_2}{C_1}$	$\frac{P_1}{P_0}$
0·1	0·92
1	0·67
2	0·60
10	0·52

It will be observed therefore that if the concentration of the permeating ions is large compared with that of the non-permeating ions the counter-pressure of Donnan will have a very important influence on the magnitude of the osmotic pressure.

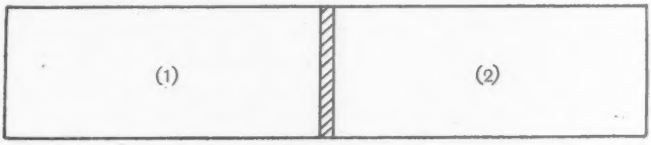

Fig. 27

Donnan and Allmand [1914] have investigated the distribution of KCl between two solutions separated by a copper ferrocyanide membrane, potassium ferrocyanide being also present in one of the solutions, and they have found the theory is supported on the whole. Bayliss [1909, 1911] has demonstrated the effect directly in experiments with congo red, H. R. Procter [1914] and Procter and J. A. Wilson [1916] have studied the gelatin-acid systems, and

S. P. L. Sörensen has very thoroughly investigated to what extent such an effect might be expected to influence his measurements of the osmotic pressure of egg albumin.

The unequal distribution is of great importance in the purification of non-permeating substances by dialysis (cf. Donnan [1911] and Sörensen [1915–1917]), and may sometimes be important in experiments to determine the active concentrations (apparent activities) of permeating substances by compensation dialysis and ultrafiltration, as has been shown particularly by Rona and György [1913] and H. J. Hamburger [1918][1].

Donnan has further shown by mathematical treatment analogous to the above that the amount of undissociated NaCl must be the same in the two solutions, which leads to the equation

$$\frac{C_{Na^{\cdot}} \cdot C_{Cl'}}{C_{NaCl}} = \text{constant}, \quad \text{.....(190)}$$

but this relation we know to be wrong (cf. chapter I). Donnan is unable to give an adequate explanation of this but it will be seen the difficulty disappears when we assume with Bjerrum that undissociated NaCl does not exist in watery solutions in the range of concentration in question. This point also brings out the superiority of Bjerrum's dissociation theory over the classical theory of Arrhenius.

In the above example of Donnan it is assumed there are no non-permeating ions in solution (2) and that the volumes of the solutions do not change (the piston in Fig. 27 being fixed). If we assume on the other hand that NaR is present in both solutions and that water can be freely interchanged between them it will be obvious without any special mathematical treatment that the solutions will be of the same constitution so that there will be no uneven distribution of $Na^{\cdot}$ and Cl'. In this case therefore we may imagine that the piston is freely movable without obstruction and that water can freely pass through it.

If there are non-permeating mols without a charge in one or both of the solutions but in a different ratio to that which the non-permeating (in this case negative) ions bear to one another, different concentrations of the non-permeating ions will be found in the two solutions when equilibrium is established and the permeating ions will consequently also be unequally distributed. A point of importance is the possibility that some force or other opposes the change in volume of the solution. In the illustration we can imagine this as happening by the piston doing work against spiral springs. In such a case the volumes, and therefore the concentrations, will not be dependent alone upon the degree of the permeating forces of the mols, and thus unequal distribution of the ions can ensue and conditions dependent thereon. If some of the non-permeating ions have a different valency to the permeating ones, a different number of permeating ions and non-permeating ions will be in

[1] Jaques Loeb has published a series of papers in the *Journal of General Physiol.* 1920–1921 on the influence of the Donnan effect on numerous factors concerned with gelatin solutions.

electrostatic equilibrium mutually. If the volumes of the solutions are only determined by the non-permeating ions and the osmotic activity of the permeating ions electrostatically balanced by the former, unequal distribution of the ions can arise.

The last complicating factor of great significance is that the solutions in question are not ideal. The values in equation (187) are therefore not the concentrations but the apparent osmotic activities so that it becomes

$$\frac{C_{\mathrm{Na}^{\cdot}(1)} \times F_a(\mathrm{Na}^{\cdot})_{(1)}}{C_{\mathrm{Na}^{\cdot}(2)} \times F_a(\mathrm{Na}^{\cdot})_{(2)}} = \frac{C_{M^{\cdot}\mathrm{I}(1)} \times F_a(M^{\cdot}\mathrm{I})_{(1)}}{C_{M^{\cdot}\mathrm{I}(2)} \times F_a(M^{\cdot}\mathrm{I})_{(2)}} = \frac{C_{A'\mathrm{I}(2)} \times F_a(A'\mathrm{I})_{(2)}}{C_{A'\mathrm{I}(1)} \times F_a(A'\mathrm{I})_{(1)}}. \quad \ldots\ldots(191)$$

Let us briefly collect the results of the above. Donnan's theory shows that the relative decrease in osmotic tension between solutions separated by semi-permeable membranes is the same for all monovalent ions, and that the relative osmotic tension of cations is the reciprocal of that of anions, and also that the relative decrease in osmotic tension of an n-valent ion is the nth root of that of a monovalent ion. If the concentrations of those permeating positive and negative ions which are balanced by non-permeating mols are different in solutions which are in equilibrium through a semi-permeable membrane, the osmotic activity of a permeating ion will be different in the solutions as first discovered by Ostwald [1890].

Let us consider a system of two homogeneous watery solutions separated by a semi-permeable membrane. The solutions contain both permeating and non-permeating ions. The non-permeating ions are distinguished by their symbols being put in parentheses, *e.g.* $(M^{\cdot}\mathrm{I}_{(1)})$, that is, the non-permeating monovalent cation in solution (1). As the following exposition is only developed as far as the present limited problems require and as experiments have not been carried out to make a general expression possible we will assume that in the solutions of permeating ions only chlorine and bicarbonate ions occur in such concentrations as are of significance for the exchange of ions, since the relations of the other ions are analogous with these two.

The following non-permeating ions are also present in the system:

$$(M^{\cdot}\mathrm{I}),\ (M^{\cdot\cdot}\mathrm{II}),\ (A'\mathrm{I}) \text{ and } (A''\mathrm{II}).$$

According to the law of equal positive and negative charges we have

$$(C_{M^{\cdot}\mathrm{I}(1)}) + (2C_{M^{\cdot\cdot}\mathrm{II}(1)}) - (C_{A'\mathrm{I}(1)}) - (2C_{A''\mathrm{II}(1)}) - C_{\mathrm{Cl}'(1)} - C_{\mathrm{HCO}'_3(1)} = 0, \quad (192)$$

$$\text{and } (C_{M^{\cdot}\mathrm{I}(2)}) + (2C_{M^{\cdot\cdot}\mathrm{II}(2)}) - (C_{A'\mathrm{I}(2)}) - (2C_{A''\mathrm{II}(2)}) - C_{\mathrm{Cl}'(2)} - C_{\mathrm{HCO}'_3(2)} = 0, \quad (193)$$

$$\text{therefore } C_{\mathrm{Cl}'(1)} + C_{\mathrm{HCO}'_3(1)} = (C_{M^{\cdot}\mathrm{I}(1)}) + (2C_{M^{\cdot\cdot}\mathrm{II}(1)}) - (C_{A'\mathrm{I}(1)}) - (2C_{A''\mathrm{II}(1)}), \quad (194)$$

$$\text{and } C_{\mathrm{Cl}'(2)} + C_{\mathrm{HCO}'_3(2)} = (C_{M^{\cdot}\mathrm{I}(2)}) + (2C_{M^{\cdot\cdot}\mathrm{II}(2)}) - (C_{A'\mathrm{I}(2)}) - (2C_{A''\mathrm{II}(2)}). \quad (195)$$

From (187) we get $$\frac{C_{\mathrm{Cl}'(2)}}{C_{\mathrm{Cl}'(1)}} = \frac{C_{\mathrm{HCO}'_3(2)}}{C_{\mathrm{HCO}'_3(1)}} = \frac{C_{\mathrm{Cl}'(2)} + C_{\mathrm{HCO}'_3(2)}}{C_{\mathrm{Cl}'(1)} + C_{\mathrm{HCO}'_3(1)}}, \quad \ldots\ldots\ldots\ldots\ldots(196)$$

and therefore

$$\frac{C_{\mathrm{Cl}'(2)}}{C_{\mathrm{Cl}'(1)}} = \frac{C_{\mathrm{HCO}_3(2)}}{C_{\mathrm{HCO}'_3(1)}} = \frac{(C_{M^{\cdot}\mathrm{I}(2)} + (2C_{M^{\cdot\cdot}\mathrm{II}(2)}) - (C_{A'\mathrm{I}(2)}) - (2C_{A''\mathrm{II}(2)})}{(C_{M^{\cdot}\mathrm{I}(1)} + (2C_{M^{\cdot\cdot}\mathrm{II}(1)}) - (C_{A'\mathrm{I}(1)}) + (2C_{A''\mathrm{II}(1)})}. \quad \ldots\ldots(197)$$

If the solutions are not ideal we obtain from (191)

$$\frac{C_{Cl'(2)}\,F_a\,(Cl')_{(2)}}{C_{Cl'(1)}\,F_a\,(Cl')_{(1)}} = \frac{C_{HCO'_3(2)}\,F_a\,(HCO'_3)_{(2)}}{C_{HCO'_3(1)}\,F_a\,(HCO'_3)_{(1)}} = \frac{C_{Cl'(2)}\,F_a\,(Cl')_{(2)} + C_{HCO'_3(2)}\,F_a\,(HCO'_3)_{(2)}}{C_{Cl'(1)}\,F_a\,(Cl')_{(1)} + C_{HCO'_3(1)}\,F_a\,(HCO'_3)_{(1)}}. \quad \ldots\ldots\ldots\ldots(198)$$

For the further treatment of the present problem it will be convenient to rearrange equation (198) thus:

$$\frac{\frac{C_{Cl'(2)}}{C_{Cl'(1)}}}{\frac{C_{HCO'_3(2)}}{C_{HCO'_3(1)}}} = \frac{F_a\,(HCO'_3)_{(2)}\,F_a\,(Cl')_{(1)}}{F_a\,(HCO'_3)_{(1)}\,F_a\,(Cl')_{(2)}}. \quad \ldots\ldots\ldots\ldots(199)$$

If solutions (1) and (2) are heterogeneous, as is the case with serum and blood cells, (199) will be further complicated as the concentrations in the solutions are then mean concentrations and we therefore have to introduce a correction for this in equation (199) (cf. chapter III). Adding the subscript (c) to the symbols relating to blood cells and (s) to those relating to serum we get

$$D' = \frac{\Phi_a\,(HCO'_3)_{(c)}\,\Phi_a\,(Cl')_{(s)}}{\Phi_a\,(HCO'_3)_{(s)}\,\Phi_a\,(Cl')_{(c)}} = \frac{\frac{C_{Cl'(c)}}{C_{Cl'(s)}}}{\frac{C_{HCO'_3(c)}}{C_{HCO'_3(s)}}}. \quad \ldots\ldots\ldots\ldots(200)$$

The symbol D' is introduced for the sake of convenience.

The value $\frac{\Phi_a\,(Cl')_{(s)}}{\Phi_a\,(HCO'_3)_{(s)}}$ can be regarded with very close approximation as being constant on account of the relatively small variations serum undergoes on changing the reaction. This is in the last resort due to the relatively small disperse phase in serum (*circ.* 8 %).

The value $\frac{\Phi_a\,(HCO'_3)_{(c)}}{\Phi_a\,(Cl')_{(c)}}$ is equal to $\frac{F_a\,(HCO'_3)_{(c)}\,\frac{100}{100 - q_{(c)}\,(1 - d\,(HCO'_3))}}{F_a\,(Cl')_{(c)}\,\frac{100}{100 - q_{(c)}\,(1 - d\,(Cl'))}}$, ...(201)

where $d\,(Cl')$ and $d\,(HCO'_3)$ are the relations between the concentrations of Cl' and HCO'_3 respectively in the protein phase and external phase of the blood cells, while the activity coefficients refer to the external phase. In accordance with the reasoning in chapter VIII we may undoubtedly regard $\frac{F_a\,(HCO'_3)_{(c)}}{F_a\,(Cl')_{(c)}}$ as constant—within the experimental error—for subsequent investigation. The value

$$\frac{\frac{100}{100 - q_{(c)}\,(1 - d\,(HCO'_3))}}{\frac{100}{100 - q_{(c)}\,(1 - d\,(Cl'))}} \quad \ldots\ldots\ldots\ldots(202)$$

must also be nearly constant as will be shown.

$q_{(c)}$ is the volume of the disperse phase expressed in vols. %. Ege [1920, 1921] has shown this is between 35 and 40 % of the blood cells. If we assume that $q_{(c)}$ in horse blood cells at p_H 6·50 is 40 it will be seen from Fig. 9 that $q_{(c)}$ at p_H 7·90 is $40\frac{86{\cdot}3}{100} = 34{\cdot}5$. $d\,(Cl')_{(c)}$ and $d\,(HCO'_3)_{(c)}$ are the relations between the solubility of the chlorine and bicarbonate ions in the protein and water phases of the blood cells. In chapter IX for the bicarbonate ion we found $d\,(HCO'_3)_{(c)}$ equal to about $\frac{1}{2}$. Provided that the chlorine ions

are not soluble in the protein phase and $d\,(Cl')_{(c)}$ therefore is 0 the variation of the conditions with the reaction will be a maximum. Substituting these values in (202) we obtain at p_H· 6·50 the value 75, and at p_H· 7·90, 79.

It will therefore be appropriate to investigate the value of D' at different reactions with the knowledge that we may expect it to be fairly constant. It is however not improbable it increases a little with decreasing apparent hydrogen ion activity.

In chapter VI there are numerous determinations of $\frac{C_{HCO'_3(c)}}{C_{HCO'_3(s)}}$ in defibrinated horse blood at room temperature and the results are given diagrammatically in Fig. 6. I have therefore undertaken some determinations of the distribution of Cl between blood cells and serum, and they are to be found in Table LXIII. The technique employed was as follows: Defibrinated horse blood was saturated in the large saturator with gas containing CO_2 (by

Table LXIII. Horse blood.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'(c)}$ calc.	Haematocrite number		Milligram equivalents of Cl in 1 litre	blood cells	D'
21	34·5	146·4	56·5	22·7?	7·40	38·5	*B*	83·8	58·6	0·46
							S	106		
						74·5	*C*	68·3	50·8	0·48
21	616·6	24·2	117·4	9·3	6·46	46·5	*B*	83·8	71·0	0·75
							S	95·0		
						75·0	*C*	75·5	69·1	0·73
21	18·9	152·4	46·8	22·2	7·69	37·0	*B*	83·8	35·7	0·32
							S	112		
						70·5	*C*	68·7	50·5	0·45
20·5	71·8	137·0	71·1	22·6	7·27	44·0	*B*	83·8	59·6	0·58
							S	103		
						70·5	*C*	71·2	57·9	0·56
20[1]	197·5	112·5	91·6	22·0	6·92	45·5	*B*	83·8	67·5	0·69
							S	97·5		
						77·3	*C*	71·6	64·0	0·66
20[1]	375·1	75·6	96·7	21·1	6·65	47·8	*B*	83·8	71·6	0·75
							S	95·1		
						81·8	*C*	74·1	69·4	0·73
20	15·0	155·9	48·2	21·3	7·81	42·0	*B*	81·9	47·4	0·44
							S	107		
						75·0	*C*	66·3	52·8	0·49
20	741·5	0·7	122·0	0·6	6·43	47·5	*B*	81·9	70·6	0·77
							S	92·1		
						72·5	*C*	72·6	65·2	0·71
19	96·4	136·6	84·0	20·9	7·20	44·0	*B*	81·9	59·6	0·60
							S	99·5		
						71·0	*C*	71·2	59·6	0·60
19	237·3	102·9	107·6	21·9	6·90	46·0	*B*	81·9	66·6	0·70
							S	95·0		
						76·3	*C*	74·5	68·2	0·72
19	30·4	150·3	58·2	21·6	7·57	42·5	*B*	81·9	53·4	0·52
							S	103		
22[2]	19·4	155·4	48·6	20·6		42·5	*B*	81·9	46·8	0·43
	19·4	155·4	59·4	0·6	7·73		*S*	108		
	19·4	155·4	37·9	39·1		77·0	*C*	63·4	50·1	0·46
22[2]	744·9	0·0	122·4	0·4		46·3	*B*	81·9	70·7	0·77
	744·9	0·0	127·4	0·0	6·47		*S*	91·6		
	744·9	0·0	116·4	—		78·0	*C*	74·6	69·9	0·76

[1] The blood was five days old and had a slightly sour smell. [2] Hirudin blood.

the method described in chapter IV) the constitution of which was determined by analysis of the spirometer gas at the conclusion of the saturation. Samples of the blood were taken to determine the amount of combined CO_2, and it was transferred to a glass cylinder, the CO_2 saturation being maintained, where it was allowed to sediment in contact with the saturating gas. The chlorine in the serum and blood cell suspension (and blood) was then estimated by means of a slightly modified titration according to Mohr and I. Bang's [1916] method. The volume of the blood cells was estimated in the usual way with the haematocrite. In estimating the chlorine, serum, blood or blood cell suspension was transferred with a 1 cc. pipette to a 50 cc. measuring flask and the pipette then washed out three times with distilled water. The flask was then filled about up to the mark with absolute alcohol with continuous shaking

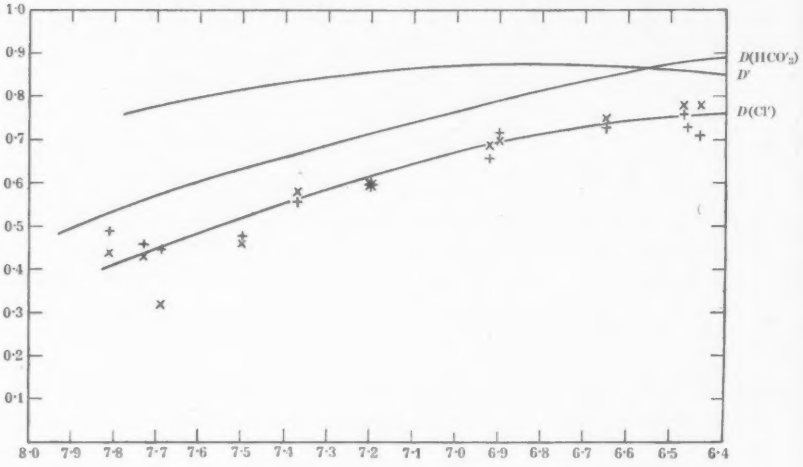

Fig. 28.

which produced a very fine precipitate. After 24 hours' standing, during which the flask was usually shaken several times, the extraction was complete and it was filled right up to the mark with absolute alcohol, whereupon the flask was rotated several times. The solution was then filtered into small Bang-flasks the funnel being covered with a watch glass to prevent evaporation and the tube passed through a cork which fitted loosely into the neck of the flask. 10 cc. of the filtrate was transferred to three flasks and titrated with $n/100$ silver nitrate with potassium chromate as indicator as described by Bang [1916].

In explanation of Table LXIII it is only necessary to state that *B* stands for blood, *S* for serum and *C* for blood cell suspension.

In Fig. 28 all the results are given, those that are obtained from blood and serum are designated by "recumbent" crosses and those from blood cell

suspensions and serum by "erect" crosses. A curve through the points was drawn freehand. This curve is marked $D\,(Cl')$. The curve from Fig. 6 (the curve showing the distribution of combined CO_2) is also given in the figure and is marked $D\,(HCO'_3)$. D' is calculated from the two curves and the result plotted as an independent curve. It will be seen that D' is about 0·85. The experiments do not justify an opinion as to whether the small variation found is a real one or due to experimental error. The result of these experiments very strongly supports the hypothesis advanced for the distribution of ions between blood cells and serum—an hypothesis which may briefly be recapitulated thus: The permeating monovalent anions distribute themselves between blood cells and serum in such a way that the relative decrease in osmotic tension is the same for them all. The relative concentration (mean concentration) of the different anions will be a function of the relative osmotic tension, of the apparent activity coefficients of the ions in the external phase, and the solubility of the ions in the internal phase.

It would have been of great help in the further elucidation of these problems had it been possible to measure the apparent (potentiometric) activity of the chlorine ions in blood cells and serum, but it is not. I shall take this opportunity of explaining why such an estimation must fail because it appears largely to have been overlooked and a knowledge of the facts is of fundamental importance in judging the value of many of the studies of Pauli and his collaborators—as is very evident from Pauli's book which appeared in 1920.

Bugarsky and Liebermann as early as 1898 attempted to measure the chlorine ion concentration in egg albumin by means of a calomel electrode. Later Manube and Matula [1913] and Blasel and Matula [1914] amongst others used the same electrode for a similar determination in protein solutions. Oryng and Pauli [1915] noticed that serum albumin combined with mercurous ions but they claim to have shown these mercurous ions were split off again at more acid reactions. I cannot see however that the authors have succeeded in demonstrating this or that it has been proved the mercurous combination is independent of the salt concentration. Pauli and his collaborators have themselves made interesting contributions to the problem of the combination by the proteins of various heavy metal ions, a combination which takes place beyond all doubt.

If the chlorine ion concentration (activity) of a solution is to be determined with the help of an electrode of the second class, be it a mercurous or silver electrode, two conditions must be fulfilled.

I. The mercurous or silver ion concentration in chloride-containing solutions must be determined solely by the activity of the chlorine ions, that is to say calomel or silver chloride must be precipitated as soon as more than a trace of mercurous or silver nitrate is added. If the mercurous or silver ions are combined in a complex manner to any solute thereby diminishing the activity of these ions to an amount smaller than necessary for the pre-

cipitation of the calomel or silver chloride, the estimation of the chlorine ion activity is not possible.

II. None of the solutes in the solution (apart from a negligible amount of chlorine ions) must be affected by the presence of mercurous or silver ions in the solution. Several investigators including Pauli and his co-workers have shown that the proteins form complex compounds with mercurous and silver ions which have an extraordinarily low dissociation tension. I have myself some experience from unpublished experiments relating to the effect of the addition of silver nitrate to blood and the following results may be stated:

(1) Considerably more silver nitrate than corresponds to $n/100$ can be added to the blood without silver chloride being precipitated.

(2) Haemolysis gradually takes place which becomes total if sufficient silver nitrate is added.

(3) At body temperature the haemoglobin is relatively slowly destroyed, and quicker in acid than in alkaline reactions.

(4) The combination of CO_2 is rather less than in the original blood at the same reaction.

(5) The O_2 combination curve at body temperature is much steeper than usual.

It will be seen that neither of the two conditions for the determination of the chlorine ion activity is fulfilled. It is appropriate to draw attention here to a fact which in a large degree obscures many of the experiments of Pauli and his collaborators and undoubtedly compromises many of their results. Pauli and his collaborators, as was generally accepted at the time, always took electrically determined apparent activity to be equivalent to the corresponding concentration, which gives rise to an error in the solutions that were poor in salt and moderately rich in protein, which in some cases may be very significant.

The experiments dealing with the distribution of chloride between blood cells and serum are further interesting as they help to throw light upon the conditions of the exchange of bicarbonate and chlorine ions. D. D. van Slyke and G. E. Cullen [1917] have tried to show that only about half the bicarbonate produced by treating serum with CO_2 (measured at roughly constant reaction) is due to exchange with chlorine ions, while L. S. Fridericia [1920] has found that the chlorine diffusion almost completely accounts for the production of bicarbonate. Van Slyke and Cullen as well as Fridericia have however disregarded the fact that the water yield of the serum to the blood cells affects their calculations. Van Slyke and Cullen found that serum from a sample of freshly-drawn blood contained

$$0{\cdot}1050n\ Cl' \text{ and } 0{\cdot}031n\ CO_2.$$

After saturation with CO_2 at atmospheric pressure the serum yielded

$$0{\cdot}111n\ Cl' \text{ and acquired } 0{\cdot}0206n\ HCO'_3.$$

If we assume the serum was concentrated 10 % the chlorine loss would be

0·0216*n*, and the bicarbonate formation 0·0266*n*. So that the difference is really much less than van Slyke and Cullen calculated.

Fridericia found that serum separated from blood at 0·08 mm. CO_2 contained 0·0992*n* Cl′ and 0·0250*n* HCO'_3, at 163 mm. CO_2.

Serum separated from blood at 162 mm. CO_2 contained

0·0814*n* Cl′ and 0·0463*n* HCO'_3, at 163 mm. CO_2.

If we assume the serum was concentrated 8 % we obtain

	Cl′	HCO'_3
Difference	− 0·0256*n*	+ 0·0183*n*

so that more chlorine has passed over than corresponds to the bicarbonate which presumably is due to experimental error.

In an experiment (Table LXIII) I found that serum separated from blood at 19·4 mm. CO_2 had a chlorine content of 0·108*n*, and that the bicarbonate content was

0·027*n* at p_H· 7·73.

The volume of the serum was 57·5 % of the blood.

Serum separated from blood at 744·9 mm. CO_2 had a chlorine content of 0·0916*n*, and a bicarbonate content of

0·0572*n* at p_H· 6·47.

The volume of the serum was 53·7 % of the blood.

In accordance with the determinations in a previous chapter horse serum will combine with about 12·5 vols. % CO_2 in changing from p_H· 7·73 to p_H· 6·47. Subtracting this (0·006*n*) and correcting for the loss of water which is 9·3 % of the original volume of the serum we get

	Cl′	HCO'_3
Difference	− 0·026*n*	+ 0·022*n*

which result like that of Fridericia's experiment gives too large a diffusion of chlorine and is probably likewise due to experimental error.

F. C. McLean, H. A. Murray and L. J. Henderson [1920] have reported experiments according to which only two-thirds of the diffused bicarbonate is accounted for by the chlorine but these experiments are only known to me through a short reference in *Medical Science Abstracts and Reviews*, **5**, p. 88, and it is not clear from this whether the authors corrected for the water loss of the serum[1].

[1] In an article which recently appeared in the *J. Biol. Chem.* (1921, **47**, 377) E. A. Doisy and E. P. Eaton have shown by accurate direct analyses that sodium and potassium do not diffuse on changing the CO_2 tension. They further showed that the diffusion of Cl′ on varying the CO_2 tension between 35 mm. and 100 mm. accurately corresponded to the diffusion of HCO'_3 (the change of volume was allowed for); on passing to very high CO_2 tensions in agreement with Fridericia and myself they found a too great diffusion of Cl′. Apart from possible experimental errors I am unable, as I have mentioned, to give an explanation of the latter phenomenon. Mukai (*J. Physiol.* 1921, **55**, 356) has likewise shown by direct analysis of the salts that the cations do not diffuse through the surface of the blood cells on changing the CO_2 tension.

Before proceeding further with the investigation of equation (198) it will be well to examine the conditions which determine the change in volume of the blood cells at different CO_2 tensions. It must however be remarked that this investigation deals with the fundamental rather than the quantitative relations of this process because the experimental material in my possession was not performed with the present purpose in view, but is a by-product of the experiments in chapter VI, and the constants used are not very accurately ascertained.

The discovery that the volume of the blood cells is increased by CO_2 treatment of the blood is credited to H. Nasse, 1874, as already stated, but he did not attempt any explanation of the phenomenon. Later it was confirmed by v. Limbeck [1894] and by Hamburger [1902]. Hamburger essayed to give a theoretical explanation of the processes on which it is based by accepting Koeppe's theory of ionic exchange between blood cells and serum. Hamburger's reasoning is as follows:

When blood is treated with CO_2 monocarbonate ions are formed in the blood cells. One of these is exchanged for two chlorine ions from the serum (plasma). In this way the osmotically active mols in the blood cells are increased and they therefore swell up, water from the serum entering them until the osmotic pressure in blood cells and serum is the same. Hamburger's view is erroneous—as is of course generally known—because monocarbonate ions are not present in sufficient quantities in the blood cells at the reactions employed and they do not increase in numbers on treating the blood with CO_2; on the contrary they decrease.

Koeppe's [1897] theory which Hamburger at any rate originally supported is in its earliest form incapable of explaining the problem as Koeppe had no idea as to how the CO_2 (according to Koeppe the monocarbonate ions) was combined.

In Spiro and Henderson's work the old theories of Zuntz [1882], Koeppe [1897] and Loewy and Zuntz [1894] were revived in a modern form. Spiro and Henderson's [1909] views were expressed in a rather different manner in conformity with the theory of ampholytes of Hasselbalch and Warburg [1918], which has been alluded to in more detail above. It is possible from the interpretation given by Loewy and Zuntz, Spiro and Henderson and Hasselbalch and Warburg of the distribution of ions in the blood to explain the change in volume of the blood cells which is determined by the reaction as Spiro and Henderson have briefly indicated.

Rich. Ege [1920], who has made the most important contribution in physiological literature since the beginning of the century to the question of the volume of the blood cells and who from nearly every point of view has decisively shown that it is a simple function of the osmotic pressure of the liquid they are suspended in, failed when it came to explaining the change of volume caused by varying reaction. He has however reported an experiment in which he added HCl to a blood cell suspension in physiological salt solution.

This caused the apparent hydrogen ion activity to rise to p_H 3·7 and the blood cells swelled 16 %. A freezing point determination of the external liquid and blood cell liquids gave the same osmotic pressure and Ege could therefore conclude the conditions obtaining in the volume change consequent on change of reaction did not differ fundamentally from those which determine the other kinds of volume change of the blood cells[1].

The mols which can exert an osmotic pressure on the membrane of the blood cells must either be incapable of passing through it, that is, be non-permeating, or they must be retained on one side of the membrane by electrostatic forces. The non-permeating mols of any importance in this respect are the cations which are all considered to be non-permeating, and protein mols (both charged and uncharged). The permeating mols which are important are consequently the anions. There is over 30 % of haemoglobin in the blood cells and about 8 % of other stroma proteins. If we assume with A. V. Hill that the haemoglobin molecules are aggregated with a mean aggregation number of 2·5, the molecular weight of the aggregated haemoglobin is about 40,000. We shall therefore not incur a large error in assuming the osmolar concentration of the blood cell proteins to be $m/100$, provided they ionise without destruction of the molecule which is now accepted by nearly everyone (cf. Pauli's [1920] criticism of T. B. Robertson's hypothesis of protein ionisation); and if the protein aggregates do not further aggregate or split up perceptibly under the given conditions the osmotic pressure exerted by the protein mols will not be altered by varying the reaction. Even if the assumptions concerning the aggregation should not turn out to be absolutely correct it will be of no consequence on account of the small osmotic activity of the proteins. On ionisation of the proteins however different amounts of permeating ions can be combined by the blood cells and it is this condition which determines the change in volume of the blood cells at different reactions. We will dwell upon this condition a little and examine it systematically on account of its great theoretical interest.

If we consider the conditions of an apparent hydrogen ion activity where the number of positive and negative charges associated with the blood cell proteins are equal, the amount of permeating anions retained in the blood cells will be equal to the amount of inorganic cations present, which in this connection may be taken to be the same as the potassium and sodium concentrations. If we now examine the conditions in a more alkaline reaction, protein anions will be formed in the blood cells in greater quantities and protein cations will have disappeared (cf. chapter XI); at the same moment there will be liberated an equivalent amount of bicarbonate ions in the form of CO_2, as protein ions now are balancing the inorganic cations corresponding to the expelled CO_2. In this way the osmolar concentration is

[1] Mukai (*J. Physiol.* 1921, **55**, 356), by determining the osmotic pressure of the blood cell fluids and serum by Barger's method, has shown that it is the same in both phases whether we are dealing with defibrinated blood treated with CO_2 or with untreated defibrinated blood.

decreased and the blood cells will shrink as water is transferred from the blood cells to the serum until the osmotic pressure is equal on either side of the blood cell membrane.

If we now consider the conditions when the reaction is more acid than before protein cations will be formed in excess of protein anions, the latter being simultaneously decreased in amount, and therefore bicarbonate ions will be held in equilibrium with the protein cations, whereby the osmolar concentration of the blood cells is increased and they will increase in size. It does not matter whether the bicarbonate ions on reaching equilibrium are exchanged with the same number of chlorine ions as the osmolar concentration is not altered in this way. The conditions are actually rather more involved than indicated here, three complications particularly being active:

I. In serum as well as in blood cells there are proteins (ampholytes), the dissociation of which depends on the reaction, which will oppose the change of volume but their action is only about $\frac{1}{40}$th of the blood cells.

II. During the ionic equilibrium an osmotic counter-pressure arises from the unequal distribution of the permeating ions as previously explained in accordance with Donnan's views. It may be surmised however that this pressure must be relatively slight.

III. The apparent osmotic coefficients should change a little on ionisation of the proteins.

I have made an attempt by a rough approximation to find out the mathematical relations of the volume change determined by the reaction and I have compared the result with the experimental one shown in Fig. 9. It should however be mentioned that the experiments in the first part of Table LXIII are in poor agreement with my other experiments in this connection but I can give no explanation of the fact. In the following no account is taken of the Donnan counter-pressure nor of any changes in the osmotic coefficient which might occur, but it is obvious that the Donnan counter-pressure will cause the volume change in the alkaline reactions to be smaller than that calculated.

The osmotic pressure on the membrane of the blood cells exerted by their fluids is made up of three components, namely the pressures due to the proteins, the cations, and the anions. If further we assume that approximately no exchange takes place between the mols of the protein phase and the external phase of the blood cells, we get this expression for the osmotic pressure in terms of normality exerted by the fluids of the blood cells,

$$(\mathbf{K}^*_{(c)} + C^*_{A'(c)})\, F_0\, (A'_{(c)}),$$

and for that exerted by the serum,

$$(\mathbf{K}^*_{(s)} + C^*_{A'(s)})\, F_0\, (A'_{(s)}),$$

where $C^*_{A'}$ only refers to the anions the concentration of which varies with the reaction, the phosphates being excluded, and where an asterisk signifies

that the symbol refers to an external phase. Further we assume with Ege that the osmotic pressure in the blood cells is the same as that in serum, which is approximately true.

On this assumption we will now consider a sample of horse blood in which the volume of the blood cells at p_H· 6·50 is 40 vols. % of the whole blood, therefore at

$$p_H\text{·}\ 6\text{·}50\ \ Q = 40,$$

and the disperse phase of the blood cells is 35 vols. % of the blood cells, therefore at p_H· 6·50 $q_{(c)} = 35$. Moreover we put at p_H· 6·50 $q_{(s)} = 9$.

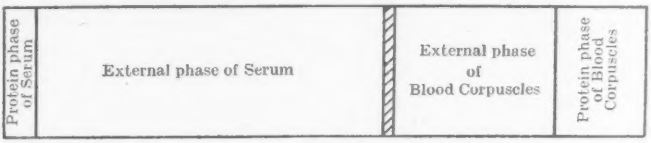

Fig. 29.

We can represent this blood diagrammatically at p_H· 6·50 by Fig. 29. The contents of the cylinder are 100 cc. of blood, the piston is the semi-permeable membrane which separates serum from the blood cells, dividing the cylinder into one compartment of 60 cc. which corresponds to the serum and another of 40 cc. which corresponds to the blood cells. From the serum space a portion 5·4 cc. is cut off which is the volume of the protein phase, and from the blood cell space a portion 14 cc. which is the volume of the blood cell proteins. The piston is assumed to be freely movable without resistance and is kept in place by the osmotic forces. We further assume that the osmotic pressure at p_H· 6·50 both in serum and blood cells corresponds to an osmotic activity of 0·3 mol. We can therefore write

$$\mathbf{K}^*_{(c)} = \mathbf{K}^*_{(s)} = 0\text{·}30 \text{ at } p_H\text{·}\ 6\text{·}50.$$

If during a change of reaction in the water phase of the blood cells a osmotic activities per litre disappear and in the water phase of the serum b osmotic activities, the osmotic activity of the blood cells will be $0\text{·}30 - a$, and of the serum $0\text{·}30 - b$.

If there is always to be the same osmotic activity in the blood cells and in the serum, water must be exchanged between them. We will call the quantity of water, expressed in vols. % of the whole blood, which must pass from blood cells to serum x and therefore

$$(0\text{·}3 - a)\frac{Q - \frac{Qq_{(c)}}{100}}{Q - \frac{Qq_{(c)}}{100} - x} = (0\text{·}3 - b)\frac{100 - Q - \frac{100 - Q}{100}q_{(s)}}{100 - Q - \frac{100 - Q}{100}q_{(s)} + x}, \quad \ldots(203)$$

from which it appears that the volume change of the blood cells is a function of the volume of the blood cells in the blood, which is easy to understand when we remember that in the limiting case where no serum is present the blood cells are quite unable to alter their volume by the appropriation of water.

In an earlier chapter we found that the combination of CO_2 in blood cells and serum can be expressed by

$$\beta = y - x p_{H'}.$$

For haemolysed horse blood with a combined O_2 content of 24·7 vols. % we found $x = 81$, and for the other haemolysate with 26·0 vols. % combined O_2, $x = 87$. In the latter haemolysate there must have been about 50 vols. % serum and since the constant x for horse serum at room temperature is about 10 we obtain $x = 80$ (*circ.*) for the share of the blood cell proteins. In pure blood cell fluids we may therefore expect a constant of about 160. The newly formed ions distribute themselves between the protein phase and the external phase but at the same time their osmotic activity is somewhat depreciated by the Milner effect. I have therefore calculated that the change in the osmotic activity of the bicarbonate ions on passing from $p_{H'(1)}$ to $p_{H'(2)}$—and consequently after exchange of bicarbonate and chlorine ions—can be expressed in terms of activity by $\frac{200\,(p_{H'(1)} - p_{H'(2)})}{2226}$.

In the case of serum I have estimated half of the compound the dissociation of which varies with the reaction comes from the phosphates, and that the change in the osmotic activity of the bicarbonate ions (and the chlorine ions exchanged with them) can be expressed by

$$\frac{5(p_{H'(1)} - p_{H'(2)})}{2226}.$$

As the variation in the combined CO_2 is a direct measure of the altered ionisation of the proteins we have, in the case under consideration,

$$a = \frac{200}{2226}(p_{H'} - 6{\cdot}50) \text{ and } b = \frac{5}{2226}(p_{H'} - 6{\cdot}50).$$

Substituting these values in (203) we get

$$x = \frac{125\,(p_{H'} - 6{\cdot}50)}{24{\cdot}2 - 2{\cdot}4\,(p_{H'} - 6{\cdot}50)}, \quad \text{.....(204)}$$

and expressing the volume of the blood cells as a percentage of the volume at $p_{H'}$ 6·50

$$x_1 = 100 - \frac{1250\,(p_{H'} - 6{\cdot}50)}{97 - 9{\cdot}7\,(p_{H'} - 6{\cdot}50)}. \quad \text{.....(205)}$$

In the first column of the following table the $p_{H'}$ values are given; in the second, the volume expressed as a percentage of the volume at $p_{H'}$ 6·50 read directly from curve II, Fig. 9; and in the last column the same amount calculated with (205).

Table LXIV.

	Volume of blood cells in % of their volume at $p_{H'}$ 6·50	
$p_{H'}$	Found	Calculated with (205)
6·50	100	100
6·80	95·2	96
7·00	92·7	93
7·20	90·7	91
7·40	89·1	88
7·60	87·8	85
7·80	86·3	81

It will be seen that the calculation indicates more shrinking of the blood cells than is actually found. In the calculation however the assumption has been made that $p_{H'}$ is the same in blood cells and serum but as shown in chapter VII this is not true. Taking this into account and using for the reaction of the blood cells the value found from Fig. 11, curve II the agreement between the found and calculated results becomes surprisingly good.

By substitution in, and modification of, equation (203) in accordance with the above it becomes

$$x_1 = 100\,\frac{14200\,(0{\cdot}090\,(p_{H'(c)} - 6{\cdot}50) - 0{\cdot}002\,(p_{H'(s)} - 6{\cdot}50))}{97 - (9{\cdot}2\,(p_{H'(c)} - 6{\cdot}50) + 0{\cdot}49\,(p_{H'(s)} - 6{\cdot}50))}. \quad \ldots\ldots(206)$$

Table LXV below gives the results of calculation with this revised equation compared with the experimentally obtained values.

Table LXV.

		Volume of blood cells in % of their volume at $p_{H'}$ 6·50	
$p_{H'(s)}$	$p_{H'(c)}$	Found	Calculated with (206)
6·50	6·49	100	100
6·80	6·78	95·2	97
7·00	6·96	92·7	94
7·20	7·13	90·7	91
7·40	7·29	89·1	90
7·60	7·45	87·8	88
7·80	7·60	86·3	86

As stated the agreement is very good, better indeed than we had a right to expect. The result is greatly in favour of the theory being the right one and that complicating factors of much importance do not play a part.

Let us revert to equation (198)

$$\frac{C_{HCO'_3\,(2)}}{C_{HCO'_3\,(1)}} \times \frac{F_a\,(HCO'_3)_{(2)}}{F_a\,(HCO'_3)_{(1)}} = \frac{C_{Cl'(2)}\,F_a\,(Cl')_{(2)} + C_{HCO'_3\,(2)}\,F_a\,(HCO'_3)_{(2)}}{C_{Cl'(1)}\,F_a\,(Cl')_{(1)} + C_{HCO'_3\,(1}\,F_a\,(HCO'_3)_{(1)}},$$

from which we approximately assume, at the concentrations employed,

$$\frac{a_{HCO'_3\,(2)}}{a_{HCO'_3\,(1)}} = \frac{C_{Cl'(2)} + C_{HCO'_3\,(2)}}{C_{Cl'(1)} + C_{HCO'_3\,(1)}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(207)$$

which in conjunction with (194) and (195) gives

$$\frac{a_{HCO'_3\,(2)}}{a_{HCO'_3\,(1)}} = \frac{(C_{M'I\,(2)}) + (2C_{M''II\,(2)}) - (C_{A'I\,(2)}) - (2C_{A''II\,(2)})}{(C_{M'I\,(1)}) + (2C_{M''II\,(1)}) - (C_{A'I\,(1)}) - (2C_{A''II\,(1)})}. \quad \ldots\ldots\ldots(208)$$

Indicating the concentrations relating to the external phases of blood cells and serum with an asterisk we get

$$\frac{a^*_{HCO'_3\,(c)}}{a^*_{HCO'_3\,(s)}} = \frac{(C^*_{M'I\,(c)}) + (2C^*_{M''II\,(c)}) - (C^*_{A'I\,(c)}) - (2C^*_{A''II\,(c)})}{(C^*_{M'I\,(s)}) + (2C^*_{M''II\,(s)}) - (C^*_{A'I\,(s)}) - (2C^*_{A''II\,(s)})}. \quad \ldots(209)$$

The numerator in the fraction on the right side of the equation stands for the difference between the non-permeating cations and anions in the external phase of the blood cells, and the denominator for the same difference in the external phase of the serum. At a reaction in the neighbourhood of $p_{H'}$ 6·50 the ionic activity in serum and blood cells is the same as regards the permeating ions. Consequently the difference referred to in the external phase of the blood cells and serum must also be of the same magnitude if (209) is

to be satisfied. And since the osmotic pressure of serum is almost identical with that of a 0·15*m* NaCl solution we can put the difference at this reaction without incurring a great error, at 0·15. If we also assume that the variation in the difference can be expressed in the same way as in the case of the volume of the blood cells we obtain

$$\frac{a^*_{HCO'_3\,(c)}}{a^*_{HCO'_3\,(s)}} = \frac{\left(0{\cdot}15 - \frac{200}{2226}(p_{H'\,(c)} - 6{\cdot}50)\right)(173 - 0{\cdot}73x)}{\left(0{\cdot}15 - \frac{5}{2226}(p_{H'\,(s)} - 6{\cdot}50)\right)(1{\cdot}54x_1 - 54)}, \quad \ldots\ldots\ldots(210)$$

where x_1 is determined by (206). The results are given in Table LXVI. In the third column the most probable values for $\frac{a^*_{HCO'_3\,(c)}}{a^*_{HCO'_3\,(s)}}$ will be found, being the reciprocals of the ratio between the apparent hydrogen ion activities of blood cells and serum.

Table LXVI.

		$\frac{a^*_{HCO'_3\,(c)}}{a^*_{HCO'_3\,(s)}}$		
$p_{H'(s)}$	$p_{H'(c)}$	Found	Calculated	$\frac{\text{Found}}{\text{Calculated}}$
6·49	6·50	(1·02)	1·00	(1·02)
6·80	6·78	0·96	0·90	1·07
7·00	6·96	0·91	0·84	1·08
7·20	7·13	0·85	0·77	1·10
7·40	7·29	0·78	0·66	1·15
7·70	7·45	0·71	0·59	1·20
7·80	7·60	0·63	0·49	1·29

According to calculation the difference in activity should be considerably greater than it is found to be, but it must be remembered the approximation we made in evolving (207) is not very good.

On the whole the results obtained, including the last part of the problem, support the theories advanced although it is quite evident several of the assumptions made are only rough approximations and are undoubtedly susceptible of considerable refinement.

Donnan [1911] has developed a simple relation between the ionic equilibrium of solutions separated by a semi-permeable membrane and the electrical potential difference between the two sides. The following is taken from Donnan and W. C. McC. Lewis.

Let us return again to the system (183) in the equilibrium (184)

R'	Na^+
Na^+	
Cl'	Cl'

Let π_1 and π_2 be the potentials of the positive electricity in solutions (1) and (2). If we transfer isothermally the very small amount of positive electricity $F\delta n$ from (2) to (1) we have the two following relations:

(*a*) The increase in free electric energy $= F\delta n\,(\pi_1 - \pi_2)$.

(*b*) $p\delta n$ mols of the Na^+ ions are transferred from (2) to (1) and simultaneously $q\delta n$ mols Cl' ions from (1) to (2), where $p + q = 1$ (p and q represent

the fraction of the total current which the respective ions have carried, or in other words they are the transference numbers of the ions). The maximal work for (*b*) is in an ideal solution given by

$$p\delta n\,\mathrm{R\,T}\log\frac{C_{\mathrm{Na^{\cdot}\,(2)}}}{C_{\mathrm{Na^{\cdot}\,(1)}}}+q\delta n\,\mathrm{R\,T}\log\frac{C_{\mathrm{Cl'\,(1)}}}{C_{\mathrm{Cl'\,(2)}}},$$

and as the electric forces balance the osmotic we obtain

$$\mathrm{F}\delta n\,(\pi_{(1)}-\pi_{(2)})=p\,\delta n\,\mathrm{R\,T}\log\frac{C_{\mathrm{Na^{\cdot}\,(2)}}}{C_{\mathrm{Na^{\cdot}\,(1)}}}+q\,\delta n\,\mathrm{R\,T}\log\frac{C_{\mathrm{Cl'\,(1)}}}{C_{\mathrm{Cl'\,(2)}}}. \quad \text{.....(211)}$$

It has been shown above that the following relation holds

$$\frac{C_{\mathrm{Na^{\cdot}\,(2)}}}{C_{\mathrm{Na^{\cdot}\,(1)}}}=\frac{C_{\mathrm{Cl'(1)}}}{C_{\mathrm{Cl'(2)}}},$$

and since

$$p+q=1, \quad \text{.....(212)}$$

we get

$$\pi_{(1)}-\pi_{(2)}=\frac{\mathrm{R\,T}}{\mathrm{F}}\log\frac{C_{\mathrm{Na^{\cdot}\,(2)}}}{C_{\mathrm{Na^{\cdot}\,(1)}}}=\frac{\mathrm{R\,T}}{\mathrm{F}}\log\frac{C_{\mathrm{Cl'(1)}}}{C_{\mathrm{Cl'(2)}}}. \quad \text{.....(213)}$$

As it has already been shown that the permeable ions distribute themselves with variable activity between blood cells and serum, a potential difference will exist between the two sides of the membrane of the blood cells. This can easily be calculated by

$$\pi_{(s)}-\pi_{(c)}=\frac{\mathrm{R\,T}}{\mathrm{F}}\log\frac{a_{\mathrm{H^{\cdot}\,(c)}}}{a_{\mathrm{H^{\cdot}\,(s)}}}=\frac{\mathrm{R\,T}}{\mathrm{F}}\log\frac{a^{*}_{\mathrm{HCO'_3\,(s)}}}{a^{*}_{\mathrm{HCO'_3\,(c)}}}. \quad \text{.....(214)}$$

At 18° we therefore have

$$\pi_{(s)}-\pi_{(c)}=0{\cdot}058\,(p_{\mathrm{H^{\cdot}_{(s)}}}-p_{\mathrm{H^{\cdot}_{(c)}}}). \quad \text{.....(215)}$$

If we now calculate the numerical values of (215) with the aid of the most probable corresponding values of $p_{\mathrm{H^{\cdot}(c)}}$ and $p_{\mathrm{H^{\cdot}(s)}}$ we obtain

Table LXVII.

$p_{\mathrm{H^{\cdot}(s)}}$	$p_{\mathrm{H^{\cdot}(c)}}$	$\pi_{(s)}-\pi_{(c)}$ volt
6·49	6·50	−0·006
6·80	6·78	+0·0012
7·00	6·96	+0·0023
7·20	7·13	+0·0041
7·40	7·29	+0·0064
7·60	7·45	+0·0087
7·80	7·60	+0·0116

The table shows that serum is positively charged against the blood cells, in other words the blood cells wander to the anode. At a reaction in the neighbourhood of $p_{\mathrm{H^{\cdot}}}$ 6·50 the blood cells change the sign of their charge. As far as one can judge, this result is in harmony with the facts but the experimental investigations into the charge of the blood cells will hardly bear a searching criticism. Although it is far from being my purpose to deal thoroughly with the views put forward concerning membrane potentials in the physiological literature (muscle and nerve physiology is particularly rich in them), it will be worth while adding a few remarks about the above calculations. Those especially interested are referred to Zangger's [1908] and Beutner's [1920] monographs and to the textbooks of physico-chemical biology, particularly Höber's [1914] and McClendon's [1917].

The above calculations are distinguished from all earlier calculations and views touching upon the membrane potentials at the surfaces of cells in that they are based on Donnan's theory. The new fact is made use of that it does not matter which permeating ion we credit with the production of the potential as all the permeating ions give rise to the same potential. A condition of the views advanced is here—as elsewhere in this work—that equilibrium is reached in the various systems, or that the equilibria at all events are so nearly approached that any deviations which occur take place very slowly. From this point of view they differ fundamentally from Beutner's ideas as he investigated the potential difference for systems of two aqueous electrolyte solutions separated by non-aqueous phases in which the cations and anions diffused with very different but nevertheless appreciable speed, so that the development of the potential difference between the watery solutions was determined by the different rate of diffusion in the intermediate liquid. Consequently the potential will not have been developed in Beutner's systems when equilibrium is reached.

As early as 1904 Höber [1904, 2; 1914] had experimentally examined the potentials on the surface of the blood cells. He used a chamber the height of which was only 100μ. It seems probable that in such a shallow chamber Höber could not ensure that the movement of the blood cells he observed under the influence of a weak current did not arise from the motion of the water along the surface of the glass since, according to Burton [1916], the stream of water is present for a distance of about 25μ. Höber conjectures from his experiments that the blood cell membrane is originally impermeable for cations and anions, and only becomes permeable for anions after CO_2 treatment. Some of H. Nasse's [1878] and Hamburger's [1902] experiments on the diffusion of chlorine in blood on blowing gas through it had however already made Höber's view improbable. Fridericia [1920] has also recently shown that it must be fallacious. I shall not further discuss Höber's contentions as I feel convinced Höber himself could not wish to retain them in their original form after the appearance of Donnan's work.

A few years ago Fåhraeus [1918] attributed the tendency of the blood cells to aggregate (and consequent rapid sinking) to a diminished potential on their surface. The experimental technique of Fåhraeus however leaves so much to be desired that the hypothesis advanced stands on insecure foundations. In a recently published very exhaustive investigation on "The Suspension-stability of the Blood," Fåhraeus [1921] seems partly to have relinquished his original view as he has shown that the substances which favour the aggregation of blood cells are the fibrinogen and the globulins of the plasma without however having elicited any definite connection between the amount of fibrinogen and the potential. H. C. Gram [1921] independently of Fåhraeus has come to the same conclusion regarding the relation between the fibrinogen of the plasma and the rate of sinking of the blood cells by the examination of a very large number of samples of human blood. Linzenmeyer's

[1920, 1921] work also supports to some extent this view, the relation of which to the haematology of bygone days is so excellently portrayed by Fåhraeus.

I have attempted to carry out new determinations of the charge on the blood cells but I encountered great experimental difficulties. Such determinations would be of considerable interest for the problems before us as we could test the accuracy of (215) with their help.

In concluding this work I would again draw attention to the very great difficulties involved in bringing the blood into equilibrium with a new CO_2 tension, a fact which we time and again encounter in the literature and which vitiates the significance of a large portion of the experiments reported there. It is generally assumed it is the mixing that is the sluggish process in the saturation of the blood cells with CO_2, and that the diffusion of CO_2 through the membrane of the blood cells, the interchanging of the ions and the ionisation of the proteins take place rapidly in comparison with it. This has however not been experimentally proved but the question of the cause of the difficulties of saturation gains physiological importance if we try to argue from the combined CO_2 at equilibrium in the blood *in vitro* to the conditions *in vivo*.

RÉSUMÉ.

I. Donnan's theory of the distribution of permeating ions in two-phase systems containing non-permeating ions is discussed and developed particularly with regard to its application to blood.

II. The distribution of chloride between the blood cells and serum of horse blood has been determined.

III. It has been proved that the distribution of bicarbonate and chlorine ions between blood cells and serum is in accord with Donnan's theory.

IV. The relation between chlorine ion and bicarbonate ion exchange has been discussed on the basis of experiments by van Slyke and Cullen, Fridericia, and the author.

V. The view that the volume change of the blood cells which is determined by the reaction is an osmotic phenomenon has been strengthened.

VI. The electric potential of the surface of blood cells has been calculated with Donnan's equation.

VII. It has been shown that it is theoretically impossible directly to determine the apparent chlorine ion activity of the blood with an electrode of the second class.

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